An Investigation of Environmental Variables Affecting Concentrations of Polycyclic Aromatic Hydrocarbons in Eastern Alaska

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AN INVESTIGATION OF ENVIRONMENTAL VARIABLES AFFECTING CONCENTRATIONS OF POLYCYCLIC AROMATIC HYDROCARBONS IN EASTERN ALASKA

A

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Abstract

Analytical methods for determining polycyclic aromatic hydrocarbon (PAH) concentrations in spruce needles were developed and evaluated. Concentrations of four PAHs (phenanthrene, anthracene, fluoranthene and pyrene) were determined in spruce needles collected near Eastern Alaska roadways. These needle concentrations were used to develop multivariate models that described the influence of climate and geographical variables on concentrations. These variables included latitude, longitude, radial distance from urban site, elevation, temperature, precipitation, ecosystem type, tree species, non-volatile extractable content of needles, and forest fire impact. The models show that three possible sources of PAHs exist in eastern Alaska, urban sites (Fairbanks, Anchorage and Valdez), ocean air, and forest fires. Distribution of PAHs away from these sources is strongly correlated with elevation. The general trend shows that PAH concentrations increase as elevation and proximity to sources decrease.

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1.0 Introduction

In the last 10 to 15 years many researchers have investigated the validity of the global distillation effect by measuring the accumulation of organic pollutant in vegetation (1-11). The theory of global distillation is based on speculation that some semivolatile organic pollutants move through the atmosphere from relatively warm source regions and condense at the colder, higher latitudes, becoming subject to accumulation into terrestrial, aquatic and marine ecosystems (1-3,12-14). Although a wide variety of organic pollutants have been studied, most attention has been given to semi-volatile lipophilic compounds. The most studied are organochlorine pesticides, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). These pollutants have detrimental health effects, so their fate in the environment is of general interest to the public.

Vegetation has become the most widely used biomonitor because it covers more than 80% of the Earth's land surface and is readily available for sampling. Also, the surface area of vegetation is 6 to 14 times greater than the land it covers (9). In addition, a lipid–rich cuticle that has been shown to be the main accumulation site of lipophilic compounds, often covers this large surface area (15). When the objective is to investigate global distribution patterns of a lipophilic pollutant, plants are clearly one of the best candidates to study.

On a global scale, this cold condensation effect is mostly determined by the general relationship of decreasing ambient temperature with increasing latitude (3). As the moving air mass cools, organic pollutants condense out. Recent studies investigated this effect in finer detail by studying the influence of other factors on smaller geographical scales. For example, the influence of elevation on distribution patterns of organochlorines in the snow pack of the Canadian Rockies (16) and PAHs in trout throughout Europe (17) have been studied. Others have looked at the relevance of localized point sources like urban centers in the United Kingdom (6) and Europe (10).

Some investigators have even looked at the relationship of socioeconomic indicators like Gross National Product per person, to distribution patterns of organochlorine pesticides (7). These results all indicate many factors other than latitude should be taken into consideration.

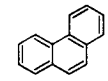
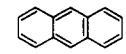
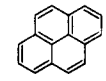
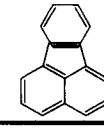
Widely varying physical properties within in the same class of pollutants further complicate the investigation of distribution patterns. For example, PAHs have a wide range of physical chemical properties. The extreme case would be vapor pressure which differs by eleven orders of magnitude from naphthalene at 10.4 Pa to dibenz[a,h]anthracene at 3.7×10^{-10} Pa at 25°C (18).

Classically, investigators have looked at the global distillation effect univariately by developing models based on concentration of an analyte or group of analytes with respect to some other single factor (1,3-7,16-17). In an attempt to account for multiple influences on analyte levels, numerous normalizing techniques have been used. In some cases, concentration is normalized to lipid content of the sample (3) or precipitation at the sample site (16). A drawback to this approach is that often the correlation between variables is poor because there are other factors acting upon the system. A more practical drawback is that looking at a multiple variable system two variables at a time is time consuming and inefficient. The overall picture can easily be clouded. Modern statistical software packages allows for multivariate modeling without having to look at each relationship one factor at a time using various normalizing techniques and data transformations.

In this study, multivariate statistics has been used to investigate geographic, climate and plant variables in relationship to PAH concentrations in Alaskan spruce needles. Four PAHs were investigated: phenanthrene, anthracene, fluoranthene and pyrene. The decision to study these four PAHs is based mainly on their suspected high level of global

distillation behavior (15) and their relatively similar physical chemical properties (Table 1.1).

Table 1.1. Physical Chemical Properties of PAHs.[a]

Compound	MW (g/mol)	B.P (°C)	P^S (Pa)	H (Pa · m^3/mol)	log K_{OA}	Structures
Phenanthrene	178.2	339	0.02	3.24	7.45	
Anthracene	178.2	340	0.001	3.96	7.34	
Pyrene	202.3	360	0.00006	0.92	8.43	
Fluoranthene	202.3	375	0.0001	1.04	8.60	

[a] P^S, H and K_{OA} are vapor pressure at 298 K, Henry's law constant and octanol-air partition coefficient, respectively (18).

The overall objective of this study was to make an exploratory investigation of distribution pattern of 3 and 4 ring PAHs in Alaskan spruce needles. Geographical, climate and plant variables were evaluated to determine their relationship to the PAH concentrations. Geographical variables include latitude, longitude, elevation, radial distance from urban sites and proximity to forest fires. Climate variables are ambient temperature and precipitation. Plant variables include species of spruce, ecosystem type, and lipid content of needles (non-volatile extractable content). A multivariate data analysis, principle component regression, was used to investigate how these variables relate to each other and to PAH level.

2.0 Method Development

2.1. Materials

2.1.1 Solvents

Hexane, EM Science, OmniSolv. 86.5% Lot # 31659.

Methylene Chloride, EM Science, OmniSolv. 99.96% Lot #36240

Acetone, EM Science, OmniSolv. 99.71% Lot #37274

2.1.2 Instruments

Hewlett Packard model 8452A Diode Array spectrometer

Hewlett Packard model 5890 GC with a model 5972 Mass Selective Detector

2.1.2.1 GC/MS Instrument Settings

The GC/MS conditions used throughout the method development procedure are shown Table 2.1.

Table 2.1. General GC/MS Conditions

Column	PTE-5(5% phenyl,95% methyl silicone), 30 m x 0.25 mm ID 0.25 μm film thickness
carrier gas	ultra high purity helium
Autosampler	Hewlett Packard 5890 Front Tray Autosampler.
injection volume	1.0 μL
injection port temp	300 °C
detector temp	300 °C

Three different GC/MS methods were used during the method development. These methods are contained within the files: HOW2.M, HOW3.M and HOW3NEW.M (Table 2.2.).

Table 2.2. GC Oven Conditions

	HOW2.M	HOW3.M	HOW3NEW.M
initial temp	125 °C	125 °C	100 °C
ramp 1	8 °/min to 240° hold 1.00 min	8 °/min to 240° hold 1.00 min	6 °/min to 240° hold 1.00 min
ramp 2	20 °/min to 280° hold 5.00 min.	20 °/min to 280° hold 5.00 min.	20 °/min to 280° hold 5.00 min.
MS mode	TIC	SIM	SIM

Method HOW2.M is a total ion current (TIC) method used to find retention times and select m/z values for the selected ion monitoring (SIM) methods. Retention times were used to develop analyzer windows, i.e., the period of time in which the mass selective detector is turned on. Outside these windows the detector is turned off to prolong the life of filaments and to lengthen time between cleaning the detector. SIM mode was used to increase sensitivity and minimize interference from the sample matrix. Analyzer windows and ions used for SIM are shown in Table 2.3. Boldface ions were used for quantitation of analytes within the particular windows. Deuterated substances were quantified using the ions in parenthesis.

Table 2.3. Selected Ion Monitoring Parameters

Analyte	Analyzer windows (min)	Ions (m/z)
Acenaphthylene	11.0-11.7	76, 151, **152**
Acenaphthene-d10 Acenaphthene	11.70-12.85	76, 153, **154**, (**164**)
Fluorene	13.75-14.80	82, 139, **166**
Phenanthrene-d10 Phenanthrene Anthracene	17.20-18.50	76, 89, **178**, (**188**)
Fluoranthene Pyrene	22.0-24.1	88, 101, **202**
Benzo(a)anthracene Chrysene-d12 Chrysene	27.70-29.0	101, 114, **228**, (**240**)

2.1.3 Standards

Glassware. All glassware was rinsed once with acetone and three times with hexane. Volumetric measurements were made with Class A volumetric glassware.

20 mg/L PAH Stock Solution. A stock solution with a target concentrations of 20 mg/L was prepared from a Supelco TCL PAH Mix (lot # LA-53928). The mixture contained 2000 μg/mL of each of the following components in methylene chloride:benzene (50:50): naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(ghi)perylene, indeno(1,2,3-cd)pyrene. 1.3474 g of this mix was diluted to 100 mL to yield a concentration of 20.0 mg/L of each component.

24.6 mg/L Internal Standard Stock Solution. A stock solution with a target concentrations of 20 mg/L was prepared from a Supelco Semivolatile Internal Standard Mix (lot # LA-60918). The mixture contained 2000 μg/mL of each of the following components in methylene chloride: 1, 4-dichlorobenzene-d4, naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, perylene-d12. 1.629 g of this mix was diluted to 100 mL with hexane to yield a concentration of 24.6 mg/L of each component.

2.1.4 Sample Collection for Method Development.

All samples were collected from Bonanza Creek (see Appendix A) wood cutting area as needed. The needle samples were collected from white spruce trees with a diameter at 1.5 m height of 15 to 25 cm. Needles were clipped from branches using scissors and placed in freezer bags. The samples were kept frozen until time of extraction.

2.1.5 Miscellaneous Materials

Chromatography Column, 45 cm glass column with i.d. of 1.0 cm

Flow cell, quartz 1 cm. cell path

Silica gel, J.T. Baker 40 μm particle size. Lot #K28678.

Florisil, MC&B 60-100 mesh Lot # FX2849109.

2.2 Optimization of Elution Profile

2.2.1 Experiment #1: Comparison of Florisil and Silica Gel.

Setup of Experiment.

The column was loaded with 3.2 g silica gel and prepped with 25 mL of hexane. The effluent end of the column was connected to a flow cell. A peristaltic pump was connected to the outlet of the flow cell. The flow rate was measured at 4.3 mL/min. A 0.4 ml aliquot of 20 mg/L PAH standard was loaded on to the column and UV/Vis spectra were collected approximately every 20 –25 seconds, providing one spectrum every 1 mL. Absorbances were monitored at 272, 320 and 334 nm with a background correction at 500 nm. See Figure 2.1 for a plot of the elution profile.

The experiment was repeated using a column loaded with 6.2 g Florisil. Absorbances were collected same as before except with a resolution of one spectrum every 2 mL. See Figure 2.2 for a plot of the elution profile.

Results.

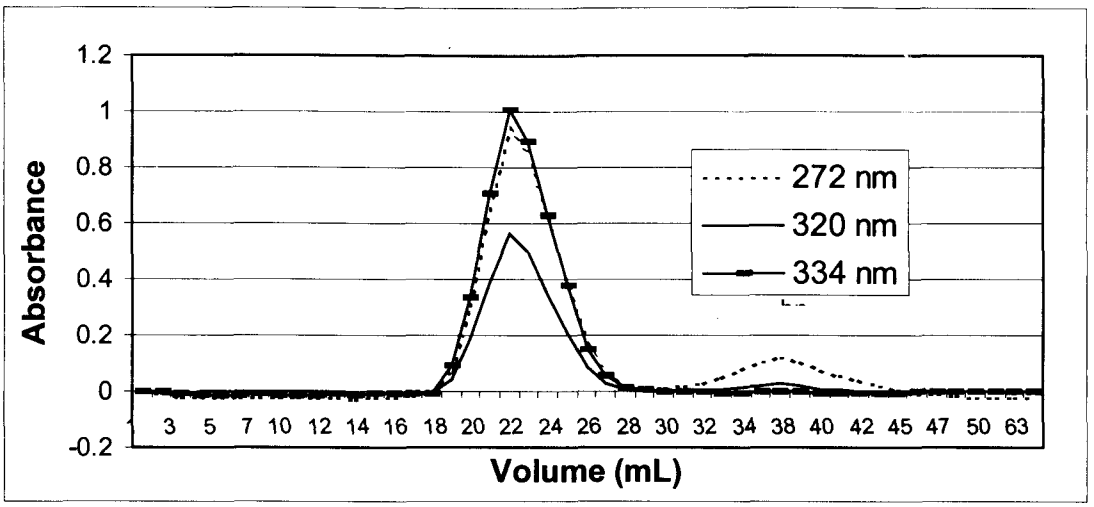


Figure 2.1. Elution profile using silica gel.

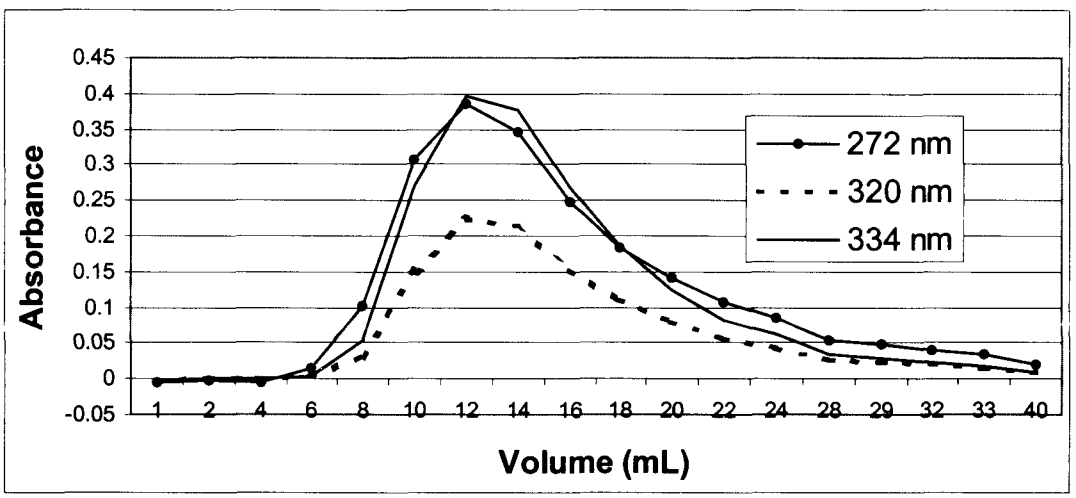


Figure 2.2 Elution profile using Florisil.

Discussion.

The silica gel gave two very distinct bands of PAH between 15mL and 50 mL while Florisil produced one broad band from about 4 mL to 30 mL. The Florisil column eluted the PAH much earlier that the silica gel column. However the PAHs showed signs of tailing on the Florisil column. In both cases the PAHs had eluted completely from the column in less than 50 mL. This experiment does not convincingly answer the question

of which solid phase to use for clean-up chromatography. It does however show that there is no clear incentive in pursuing Florisil and there should be no detrimental consequences to focusing on optimizing a silica gel clean-up chromatography scheme.

2.2.2 Experiment #2: Defining Fractions on Silica Gel.

Setup of Experiment.

The column was loaded with 4.0 g silica gel and prepped with 25 mL of hexane. Flow was controlled by N_2 pressure. Head pressure was adjusted to give a column flow rate between 1.5 and 2 mL/min. The column was loaded with 500 µL of 1 mg/L PAH standard and eluted with 45 mL hexane and then 50 mL 1:1 CH_2Cl_2:hexane. Five milliliter fractions were collected in 5 dram vials. Each fraction was then evaporated to half volume, transferred to a conical vial, further evaporated to 0.5 mL under a steady flow of N_2, and transferred to a GC/MS autosampler vial. Each fraction was then analyzed for PAH content using GC/MS method HOW3.M. Quantitation of analytes was achieved using a calibration design using 50, 200, 500, 600, and 1000 µg/L PAH standards prepared by serial dilution from 20 mg/L stock. See Figure 2.3 for results. Recoveries of the analytes were also determined. The 50 µg/L calibration standard was run in triplicate to give an estimate of the statistical instrument method detection limit (MDL). See Table 2.4 for the MDL results and Table 2.5 for the percent recovery results.

Results

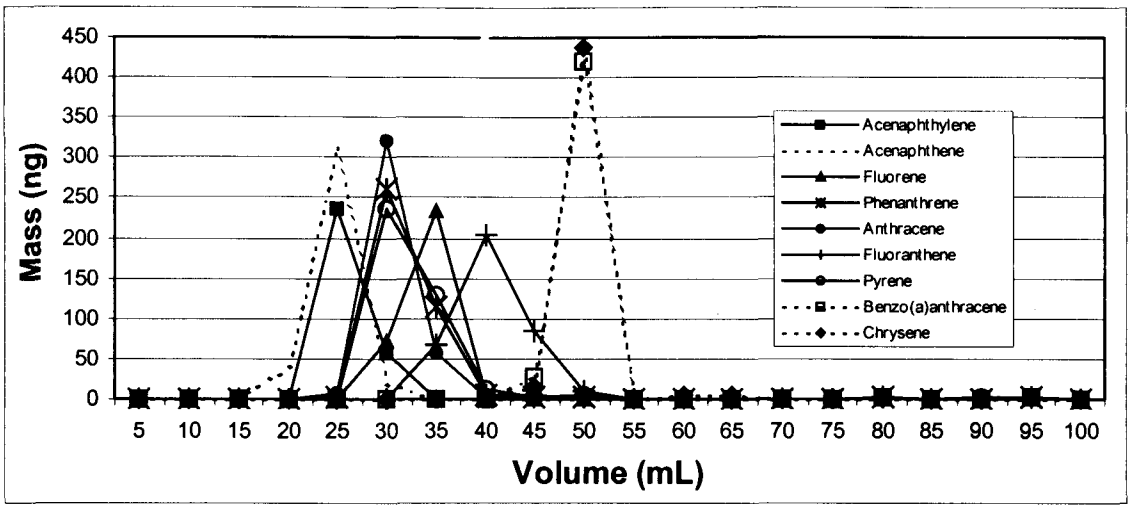


Figure 2.3. Elution profile for experiment #2. Mobile phase changed from hexane to 1:1 CH_2Cl_2:hexane at 45 mL.

Table 2.4. MDL for PAH analyte list

Analyte	MDL(µg/L)
Acenaphthylene	6
Acenaphthene	6
Fluorene	6
Phenanthrene	7
Anthracene	5
Fluoranthene	8
Pyrene	7
Benzo(a)anthracene	14
Chrysene	16

MDLs were estimated by multiplying the standard deviation by a value of 3.

Table 2.5. Recovery of PAH Analytes Using Silica Gel

Analyte	Recovery (%)
Acenaphthylene	60
Acenaphthene	75
Fluorene	64
Phenanthrene	78
Anthracene	80
Fluoranthene	74
Pyrene	79
Benzo(a)anthracene	90
Chrysene	94

Recoveries were calculated by summing the mass of analyte in each fraction and dividing by the mass of analyte loaded onto the column.

Discussion.

The objective of these elution profile experiments was to determine if all the PAH's could be eluted with a moderate volume of hexane and separated from more polar chlorinated organics. While most of the PAH analytes elute in the hexane fraction, the larger PAH compounds, benzo(a)anthracene and chrysene, elute just after the change in solvent. Figure 2.3 also indicates that fluoranthene partially elutes into the second fraction.

The MDL calculation was an attempt to get a preliminary estimate of the instrument detection limit. From Table 2.4 about 6 ng is a good estimate of a MDL with the two largest PAH compounds having a MDL of about 15 ng. Better estimates of the MDLs could possibly be achieved by analyzing a smaller standard with greater replication. The recoveries were also encouraging. They ranged from 60 to 94%. There also seemed to be a trend of increasing recovery with decreasing volatility of the compound. This is most likely the result of loss of analyte in the evaporating steps prior to GC/MS analysis and can be accounted for by addition of surrogates/internal standards.

2.2.3 Experiment #3: Elution Profile of Spiked Needle Sample.

Setup of Experiment

Extraction

Needles were ground with a coffee bean grinder. Ten grams of ground needles were extracted with 75 mL hexane using a Soxhlet apparatus. The Soxhlet apparatus consisted of a 125 mL round bottom flask, Soxhlet extractor with glass thimble, and a cold water condenser. Heat was applied to round bottom flask with a hot water bath. The extraction went for six hours with an extractor fill and drain time of 2 minutes. The extract was vacuum filtered through a fritted glass funnel and returned to the 125 mL round bottom flask. The funnel and filtration vessel were then rinsed three times with 15 mL of hexane. The rinsings were added to the extract. The extract was roto-evaporated to about 3 mL, transferred to a conical vial, and further evaporated to 1 mL under a steady flow of N_2.

Clean-up Chromatography.

The column was loaded with 4.0 g silica gel and prepped with about 25 mL of hexane. Pressure was applied to the top of column using compressed N_2 gas, to give a flow rate between 1.5 and 2 mL/min. The column was loaded with 1 mL of the above extract spiked with 0.5 mL of 1 mg/L PAH standard. The column was then eluted with 45 mL hexane and then 45 mL 1:1 CH_2Cl_2:hexane. Five milliliter fractions were collected in 5 dram vials. Each fraction was evaporated to half volume, transferred to a conical vial, further evaporated to 0.5 mL under a steady flow of N_2 and then transferred to a GC/MS autosampler vial. Each fraction was then analyzed for PAH content using GC/MS method HOW3.M. Quantitation of analytes was achieved using a two-point calibration curve determined from 200 and 600 µg/L PAH standards. See Figure 2.4 for results. Recoveries of the analytes were also determined. The 600 µg/L calibration standard was analyzed four times to give an estimate of the GC/MS reproducibility in terms of relative standard deviation (RSD). See Table 2.6 for the recovery results and Table 2.7 for RSD results.

Results

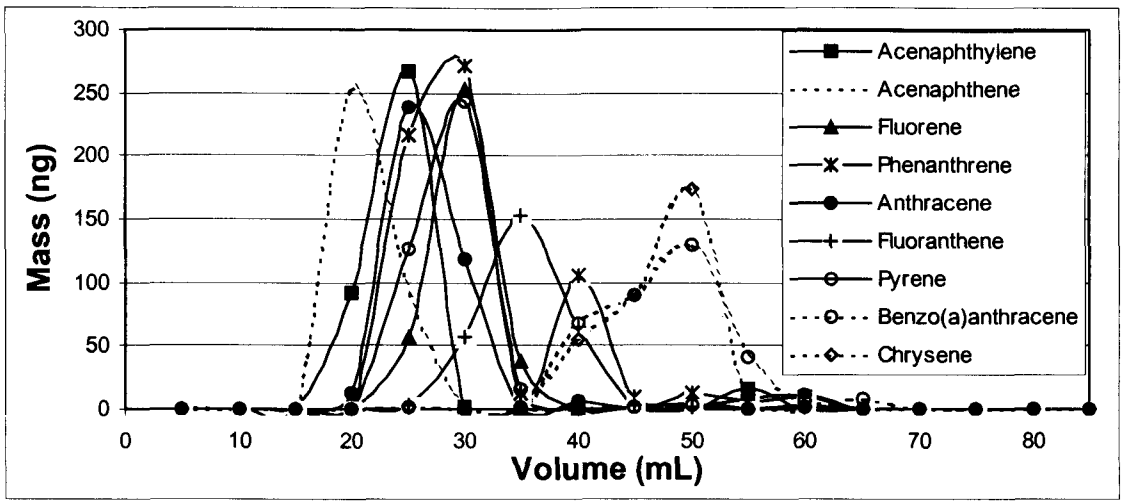


Figure 2.4. Elution Profile for spiked spruce needle sample.

Table 2.6. Recovery of PAH Analytes from Spiked Sample.

Analyte	Recovery (%)
Acenaphthylene	75
Acenaphthene	70
Fluorene	71
Phenanthrene	129
Anthracene	80
Fluoranthene	57
Pyrene	78
Benzo(a)anthracene	69
Chrysene	64

The recoveries were calculated the same as in elution profile experiment #2.

Table 2.7. RSD for PAH GC/MS Analysis

Analyte	RSD (%)
Acenaphthylene	6
Acenaphthene	5
Fluorene	6
Phenanthrene	5
Anthracene	7
Fluoranthene	7
Pyrene	6
Benzo(a)anthracene	17
Chrysene	16

The RSD calculations are based on the standard deviation of four replicate GC/MS analysis of a 600 μg/L PAH Standard.

Discussion

The goal of having all the PAH analytes elute within the hexane fraction does not look realistic under the current scheme. Figure 2.4 together with results from section 2.2.2 show convincingly that benzo(a)anthracene and chrysene elute within the 1:1 CH_2Cl_2:hexane fraction. Perhaps increasing the hexane fraction to 100 mL would allow these larger PAH compounds to elute well before the start of the 1:1 CH_2Cl_2:hexane fraction. However, this is not consistent with the overall goal of simplifying and shortening the clean up chromatography procedure without jeopardizes the efficiency of the procedure. An alternative would be to skip the hexane fraction and just elute with 1:1 CH_2Cl_2:hexane.

The recovery for the spiked sample (Table 2.6) was similar, within the 60 to 90 % range, to recoveries from previous experiments (Table 2.5). The exception was phenanthrene, which was much higher and is the result of analyte present in the needle sample. Benzo(a)anthracene and chrysene had considerably lower recoveries than earlier experiments (Tables 2.5 and 2.6). This could be the result of two things: sample matrix causing interference or, more likely, the GC/MS retention times were longer than in the previous experiments. A pressure leak in the GC injection port caused the retention times

to shift outside the MS analyzer windows in some of the chromatograms. The shift in retention times was no larger than 15 seconds, however the analyzer window was not wide enough to allow for this shift. The GC/MS program HOW3 was modified to have broader analyzer windows.

The GC/MS instrument RSD was also determined (Table 2.7). With the exception of benzo(a)anthracene and chrysene the instrument RSD was between 5 and 7 %. Benzo(a)anthracene and chrysene had much larger RSDs of 16 and 17%. This indicates that the GC/MS is not performing optimally for these compounds. The addition of internal standard/surrogate would most likely clear up this problem.

2.2.4. Elution Profile Using a Hexane/Methylene Chloride Mobile Phase

Setup of Experiment

Extraction.

Four needle samples and one method blank were extracted in the same manner as the previous elution profile experiment. All five extractions had 250 µL of 307 µg/L PAH internal standard added to the needles in the Soxhlet thimble prior to extraction. Two of the extractions also were spiked with 250 µL of 250 µg/L PAH standards prepared by serial dilution from 20 mg/L stock.

Clean-up Chromatography

Columns were prepared as in section 2.2.3. Extracts were loaded onto the columns and then eluted with 25 mL of 1:1 CH_2Cl_2:hexane. All but the spiked extracts were collected in one complete fraction of 25 mL. The first 15 mL of the spiked extracts were collected as one fraction with subsequent collection of 5 mL fractions. Each fraction was then evaporated to half volume, transferred to a conical vial, further evaporated to 0.3 mL under a steady flow of N_2 and then transferred to a GC/MS autosampler vial. Each fraction was then analyzed for PAH content using GC/MS method HOW3.M. Quantitation was achieved using a single-point calibration curve determined from

duplicate 500 μg/L PAH standards. Table 2.8 shows the recoveries for the spiked samples. Recovery was calculated by subtracting the average value of the unspiked sample from the spiked sample.

Results

All analytes eluted within the first 15 mL fraction. Table 2.8 shows the recoveries of the analytes.

Table 2.8. Recoveries from Spiked Samples

	Recovery (%)	
	Spiked Sample # 1	Spiked Sample # 2
Acenaphthylene	146	115
Acenaphthene	120	108
Fluorene	117	86
Phenanthrene	113	73
Anthracene	124	104
Fluoranthene	114	101
Pyrene	112	100
Benzo(a)anthracene	128	104
Chrysene	118	103

Discussion.

All analytes eluted within the first 15 mL, simplifying the elution scheme. The goal of having a single fraction to analyze has been accomplished. The recoveries of the analytes indicate the lack of matrix effects. Addition of internal standard to the samples seems to increase the consistency of recoveries among the different analytes.

2.3 Optimization of Extraction Procedure.

2.3.1 Experimental Design #1: Optimization of Soaking Extraction.

Design of Experiment #1

This experiment was designed to investigate the effects of seven variables on the extraction procedure. Each experiment was set up according to the design described in

Table 2.9. The 2^{7-3} fractional design was generated using Design-Expert Version 5.0.7 (Stat-Ease Corporation, Minneapolis, MN).

Table 2.9. Fractional Factorial Design for Experimental Design #1: 7 variables & 16 experiments

Exp. #	Variable A Grinding	Variable B Drying	Variable C Extraction Temperature (°C))	Variable D Extraction Time (hr.)	Variable E % Acetone	Variable F Solvent Volume (mL)	Variable G Spin Bar
1	No	No	4	24	20	100	No
2	Yes	No	4	2	0	100	Yes
3	No	Yes	4	2	20	75	Yes
4	Yes	Yes	4	24	0	75	No
5	No	No	20	24	0	75	Yes
6	Yes	No	20	2	20	75	No
7	No	Yes	20	2	0	100	No
8	Yes	Yes	20	24	20	100	Yes
9	Yes	Yes	20	2	0	75	Yes
10	No	Yes	20	24	20	75	No
11	Yes	No	20	24	0	100	No
12	No	No	20	2	20	100	Yes
13	Yes	Yes	4	2	20	100	No
14	No	Yes	4	24	0	100	Yes
15	Yes	No	4	24	20	75	Yes
16	No	No	4	2	0	75	No

This design was performed in two stages. The results of experiments 1 through 8 guided the foldover experiments, 9 through 16.

Extraction Setup.

Drying. Twenty-five grams of whole needles were dried for 18 hours at 50 °C. The cooled needles were then treated according to the design.

Grinding. A mortar and pestle were used to grind the needles. The needles were first frozen with liquid nitrogen and quickly ground, except for those used in experiments 2 and 6. The needles for these two experiments were ground unfrozen, which proved to be very inefficient and led to the addition of a freezing step to the procedure.

Extraction. Twenty-five grams of needles were placed in 250 mL ground glass joint reagent bottles. The needles were then covered with the desired volume of solvent. Samples with one inch magnetic spin bars were extracted atop a magnetic stirring. The warm extractions were done at room temperature and the cold extractions were done at 4°C.

Clean–up Chromatography

Extracts were transferred to tared 250 mL round bottom flask and roto-evaporated to dryness. The flask was then reweighed. This dried extract was then dissolved with 1 mL of hexane and transferred to a prepped silica gel column. The flask was rinsed twice more with 1 mL of hexane with the rinse being added to the column. The column was eluted with 35 mL of 1:1 CH_2Cl_2:hexane. This entire fraction was collected in a tared 50 ml round bottom flask. As before the flask was roto-evaporated to dryness and weighed again.

Analysis.

The dry extracts were dissolved in 10 mL of hexane and analyzed by GC/MS using HOW3.M. The peak area for an analyte was normalized by dividing each analyte's peak area by the largest peak area for that analyte in the experiment. These data were then summed for each sample and multiplied by 1000 to yield what was labeled as "sum PAH signal".

Results

Table 2.10 shows masses and summed PAH peak areas for the 16 experiments.

Table 2.10. Response List for Experimental Design #1.

exp. #	mass extracted (mg)	mass left after clean up (mg)	sum PAH signal
1	275	7	291
2	377	93	250
3	202	17	238
4	431	93	245
5	173	15	409
6	591	75	554
7	103	28	121
8	597	224	376
9	385	91	518
10	165	13	431
11	202	2	464
12	110	25	160
13	288	76	408
14	66	26	401
15	211	47	349
16	30	17	213

Table 2.11 shows the results of this analysis in terms of standardized effects of each factor. Figures 2.6 through 2.8 provide a visual display of the normal probability plots for the experiments.

Table 2.11. Effects List for Optimization of Extraction Experiment #1.

Factor	Effects		
	mass extracted (mg)	mass after clean-up (mg)	sum PAH signal
A	224	69	113
B	34	36	6
C	56	12	80
D	4	1	63
E	84	15	23
F	-21	14	-61
G	5	28	3
AB	47	31	-24
AC	61	9	85
AD	-54	7	**-137**
AE	-11	21	29
AF	-17	8	19
AG	10	24	-41
BD	66	35	-21
Lenth's ME*	132	59	115

*Lenth's Margin of Error.

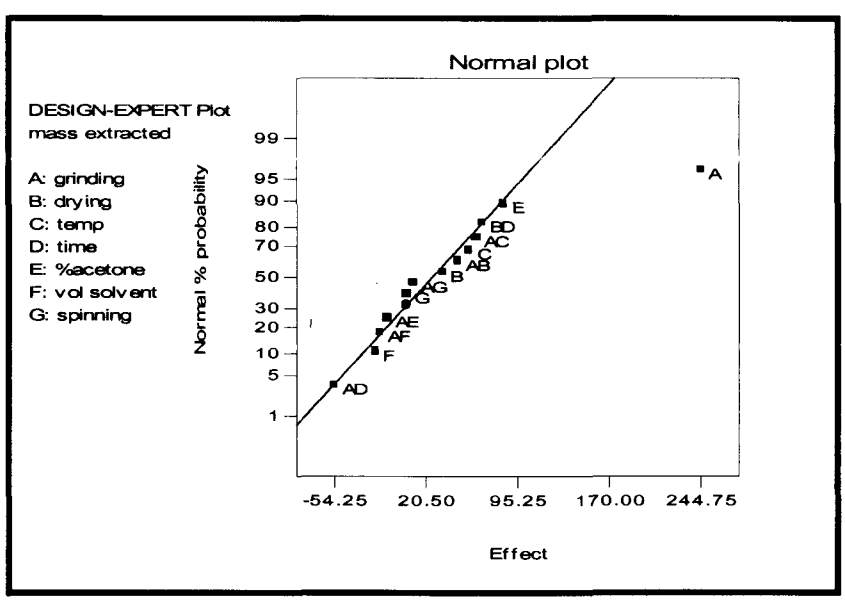


Figure 2.5. Normal probability plot of mass extracted.

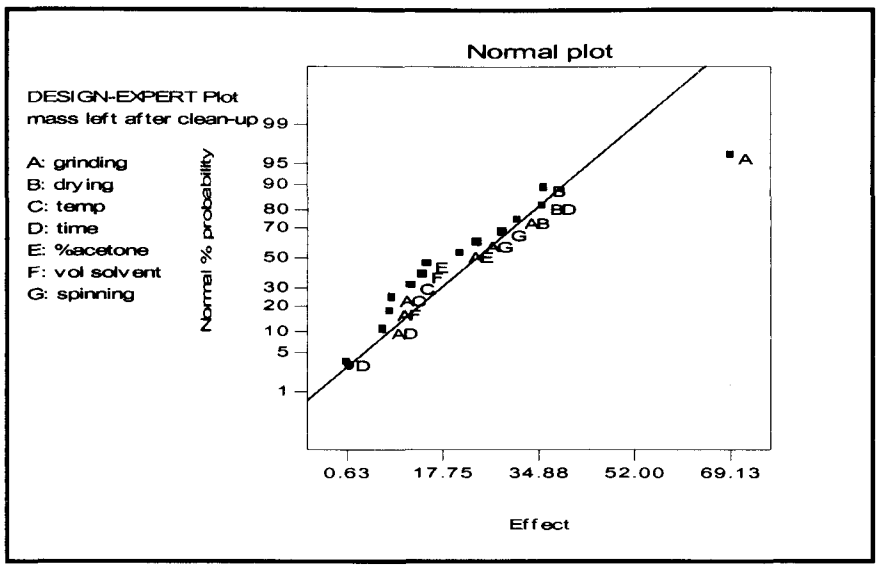


Figure 2.6. Normal probability of mass left after clean-up.

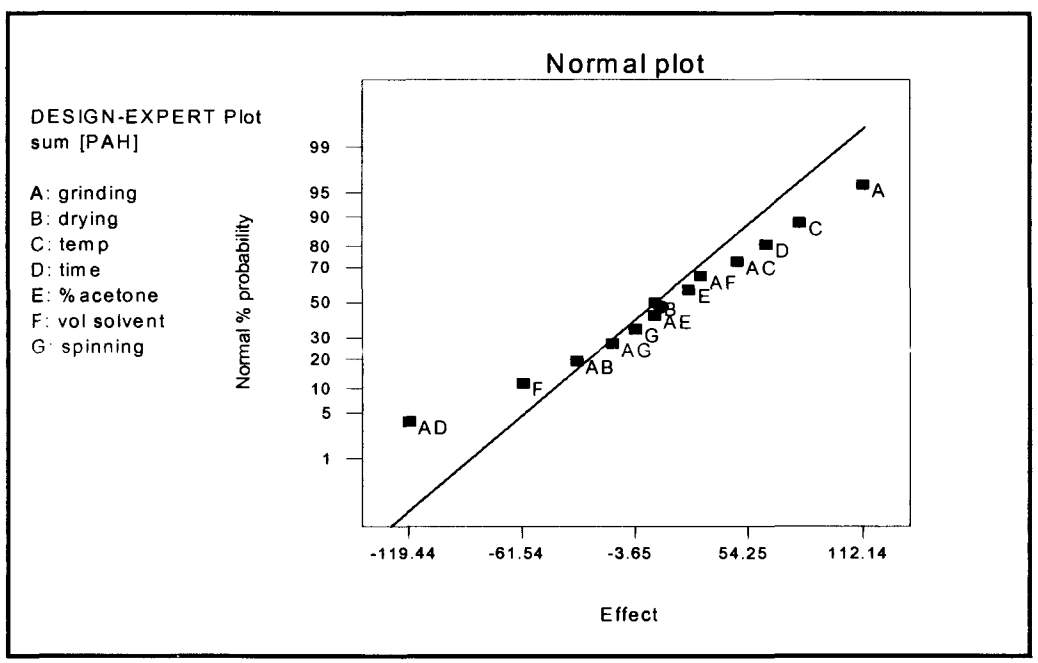


Figure 2.7. Normal probability plot of sum PAH signal.

Discussion.

The interpretation of these results is most easily done by inspecting the normal probability plots (Figure 2.5-2.7) and comparison of the Lenth's ME (19) value to the list of factor effects in Table 2.11. The normal probability plots visually show which factors had a statistically significant effect on the response. Factors that lie to the extreme left or right can be considered important. The Lenth's ME is a calculated absolute significance level based on a studentized absolute median of the calculated effects. The results clearly show that grinding is a significant variable in both the mass extracted from the needles and the amount of this mass remaining after clean-up chromatography. The idea that grinding the needles prior to extraction would increase the mass extracted makes intuitive sense. The surface area to solvent ratio increases greatly and therefore it seems logical that the extract mass would be greater. Also by grinding you are possibly exposing parts of the needles to solvent that could not otherwise be effectively exposed to it. The design results for PAH content extracted is not as clear. The normal probability plot shows that factors A (grinding) and AD have significant effects. The Lenth's ME indicates that just AD is active and perhaps A is also. Since this is a fractionated design, factor AD is aliased with two other interactions. Factor AD is actually equivalent to the sum of the AD (grinding:time), CG(temp:spinning), and EF(%acetone:solvent volume) interactions. Since factor A, grinding, shows signs of being active the more likely scenario is the grinding and time interaction. Table 2.12 shows the average sum PAH signal for the design in regards to the grinding and time variables.

Table 2.12. Average PAH Signal for Grinding and Time Variables.

	No grinding	grinding
24 hr extraction	383	358
2 hr extraction	181	432

Table 2.12 indicates that when whole needles are soaked for only two hours, the PAH content is only partially extracted. Either grinding the needles or soaking them longer

completes the extraction. Grinding the needles allows for a much shorter extraction time, which reduces the sample preparation time considerably.

2.3.2 Experimental Design #2: Comparison of Soaking and Soxhlet Extraction Techniques.

Design of Experiment #2

Design Expert Version 5.0.7 was used to design a 2^4 factorial experiment to study four extraction variables. These were the two active variables, grinding and extraction time, % acetone in the extraction solvent, and the method of extraction, either soaking or Soxhlet. The single block experimental design is in Table 2.3.

Table 2.13. Factorial Design for Experimental Design #2: 4 variables & 16 experiments

Experiment #	Variable A Extraction Type	Variable B Grinding	Variable C Time (hr.)	Variable D % Acetone
1	soaking	no	2	0
2	Soxhlet	no	2	0
3	soaking	yes	2	0
4	Soxhlet	yes	2	0
5	soaking	no	6	0
6	Soxhlet	no	6	0
7	soaking	yes	6	0
8	Soxhlet	yes	6	0
9	soaking	no	2	20
10	Soxhlet	no	2	20
11	soaking	yes	2	20
12	Soxhlet	yes	2	20
13	soaking	no	6	20
14	Soxhlet	no	6	20
15	soaking	yes	6	20
16	Soxhlet	yes	6	20

This design was performed in a single block of experiments.

Extraction Setup.

Grinding. An electric coffee bean grinder was used to grind needles. The needles were taken from the freezer and ground for approximately 30 seconds.

Extraction. For the soaking extractions, 10.0 g of needles were placed in 125 mL round bottom flask. The needles were then covered with 50 mL of either hexane or 1:4 acetone:hexane. For the Soxhlet extractions, 10 g of ground needles were extracted using a Soxhlet apparatus. The Soxhlet apparatus consisted of a 125 mL round bottom flask, Soxhlet extractor with glass thimble and a 5°C water condenser. Needles were extracted with 50 mL of the desired solvent. Heat was applied to the round bottom flask with a heating mantle adjusted to give an extractor fill and drain time of 2 minutes. All experiments were extracted for the desired time of either two or six hours. After the extractions were completed the extracts were vacuum filtered through a fritted glass funnel and returned to the 125 mL round bottom flask. The funnel and filtration vessel were then rinsed three times with 15 mL of hexane. The rinsings were added to the extract.

Clean–up Chromatography

The extracts were roto-evaporated to about 3 mL, transferred to a conical vial and further evaporated to 1 mL under a steady flow of N_2. The concentrated extracts were then loaded into a prepared silica gel column. The flask was rinsed twice more with 1 mL of hexane, with the rinse being added to the column. The column was eluted with 45 mL of hexane and then 10 mL 1:1 CH_2Cl_2:hexane. The eluate was collected in two fractions, one for each solvent. The fractions were roto-evaporated to approximately 3 mL and then transferred to a 3.0 mL conical vial. The fractions were then further concentrated by evaporating down to 0.3 mL under a steady stream of N_2. The volume of each fraction was then measured using a 500 µL gas-tight syringe and transferred to a GC/MS autosampler vial.

Analysis.

Each fraction was analyzed for PAH content using GC/MS method HOW3.M. A five-point calibration curve was determined from 41.7, 83.3, 125, 250, and 500 ug/L PAH standards. See Table 2.14 for results.

Table 2.14. GC/MS Results for the Factorial Design #2 Experiments.

	Mass of Analyte in Sample (ng)									
Exp. #	Acenaph-thylene	acenaph-thene	fluorene	phen-anthrene	anthracene	fluor-anthene	pyrene	benzo(a) anthracene	chrysene	Sum PAH
1	0.6	22.1	25.7	75.0	2.7	16.5	19.5	2.1	2.6	167
2	0.1	10.0	23.6	75.7	1.9	19.5	18.9	0.4	0.5	151
3	0.1	5.5	10.6	52.0	1.3	16.1	16.2	0.3	0.3	102
4	0.5	6.9	25.5	93.6	2.3	13.8	27.3	0.3	0.4	171
5	0.4	30.9	28.9	86.3	2.4	14.4	22.0	0.3	0.3	186
6	0.1	1.6	7.3	31.5	0.8	7.1	6.6	0.2	0.4	56
7	0.0	7.8	12.3	43.3	1.8	14.0	18.1	0.6	1.9	100
8	0.2	3.4	14.7	59.0	1.7	11.7	15.7	0.5	1.0	108
9	0.1	11.4	23.2	68.2	2.7	17.4	17.1	0.4	2.6	143
10	2.0	9.8	18.0	37.0	1.0	11.3	14.4	0.2	1.6	95
11	1.2	16.5	29.9	105	1.6	5.9	30.6	0.4	0.4	191
12	1.6	18.2	31.0	101	1.6	32.9	40.4	0.4	0.3	227
13	0.1	6.4	13.5	33.4	0.5	12.0	15.2	1.3	3.2	86
14	0.3	7.3	20.5	72.1	3.2	22.1	20.6	0.1	0.5	147
15	0.7	3.4	13.9	48.5	0.9	13.1	14.2	0.4	0.4	96
16	0.1	10.9	28.2	104	3.0	29.0	37.1	0.1	0.2	213

The results from Table 2.14 were analyzed to see which variables were active using Stat-Ease software for factorial design analysis. The analytes, acenaphthylene, anthracene, benzo(a)anthracene, and chrysene were not analyzed individually in the factorial design because of the very low concentrations however they were included as part of the Sum PAH concentrations. See Table 2.15 for the list of effects.

Table 2.15. List of Effects for Each Response.

Term	acenaphthene	fluorene	phenanthrene	fluoranthene	pyrene	Sum PAH
A	-4.5	1.3	7.8	4.8	3.5	11.5
B	-3.4	0.7	15.9	2.0	8.2	21.6
C	-3.6	-6.0	-16.1	-1.2	-4.3	-31.7
D	-0.5	3.7	6.6	3.8	5.6	19.2
AB	6.0	6.8	19.5	4.8	6.9	45.9
AC	-1.8	-0.8	6.0	-0.6	-0.9	1.4
AD	6.6	3.0	7.0	7.0	5.4	30.1
BC	-1.8	-0.9	-8.0	1.0	-3.0	-12.2
BD	6.9	6.3	21.0	2.5	5.6	42.3
CD	-3.4	-0.5	2.9	3.4	0.5	2.7
ABC	1.8	1.0	2.4	-2.1	0.8	3.8
ABD	-3.6	-3.4	-8.4	4.9	0.7	-10.9
ACD	3.9	7.2	26.3	1.9	6.2	46.1
BCD	-1.4	-1.9	-5.4	-1.5	-3.0	-13.9
ABCD	-1.0	-0.8	-5.0	-4.7	0.5	-10.7
Lenth's ME	13.1	7.4	30.1	9.7	13.6	53.7
Minimum Detectable Significant Effect	6.7	9.5	33.0	8.6	8.0	58.2

Discussion

It is clear from Table 2.15 that none of the factors investigated in this experiment are significant in term of PAH concentrations. None of the factors gave effect values that were greater than the Lenth's ME values. Also, none of the effects were convincingly greater than the minimum detectable significant effect. This is an alternative significance level based on a studentized pooled effect of the three and four factor interactions. By pooling these effects an estimate of the random error of the design is estimated. Further, studentizing this value gives an estimate of a minimum significance level based on a 95 % confidence level. An evaluation of residuals and outliers led to the possibility of removing some data, however this did not change the overall interpretation of the results. Based on statistical interpretation, whether the ground or whole needles were extracted by soaking or Soxhlet in 20 % acetone:hexane or just hexane for two hours or six hours the PAH concentrations were the same. This interpretation implies that variability in analyte concentrations among the 16 experiments is due to random effects. However, it

is unlikely that the large ranges in concentrations evident in Table 2.14 can be entirely the result of random effects. Biological samples by nature are highly variable as evident from the inconsistencies of the magnitude of concentrations within the same experiment. So, it is believable that random effects could account for a large part of the variability, possibly even a factor of two difference in concentrations. Even still there seems to be an effect not being accounted for by this design that is causing variability.

In summary, this design indicates that the four parameters manipulated do not have any noticeable effect on the concentration of PAH extracted.

2.4 Homogeneity of Tree Experiment.

Sample Collection.

Both old and first year growth needles were collected from the top (28 –30 meters), middle (15-16 meters), and bottom (2-3 meters) branches of a white spruce. In total, six samples were collected. Samples were stored in a freezer until time of extraction.

Extraction

Needles were ground with a coffee bean grinder. Fifty grams of ground needles were extracted in 200 mL of hexane for 60 hours. Extractions were performed in duplicate. After the extraction was completed the extracts were vacuum filtered through a fritted glass funnel. Extraction vessels and funnel were then rinsed three times with 30 mL of hexane. The rinsings were added to the extract. The extracts were transferred to tared 250 mL round bottom flask and roto-evaporated to dryness. The flasks were then weighed again to determine the dry mass of the extract. The dry mass of the extracts were then analyzed by two factor ANOVA with replication. The results of this are in Table 2.16.

Clean–up Chromatography

The columns were prepared the as in section 2.2.2. The extracts were dissolved with 1 mL of hexane and loaded onto the column. The flasks were rinsed three times with 0.5 mL hexane and also loaded on to column. The columns were eluted with 35 mL hexane and then 35 mL 1:1 CH_2Cl_2:hexane. The hexane and 1:1 CH_2Cl_2:hexane eluate were collected separately in 50 mL round bottom flask. Each fraction was then evaporated to approximately 1 mL under a steady flow of N_2 and then transferred to a GC/MS autosampler vial.

Analysis

Each fraction was analyzed for PAH content using GC/MS method HOW3.M. The samples were quantified using a five-point calibration curve determined from 50, 100, 200, 600, and 1000 µg/L PAH standards prepared by serial dilution from 20.0 mg/L PAH stock solution. Phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene and chrysene were the only analytes quantified. Fraction concentrations were summed to give a sample concentration. Table 2.17 lists the sample concentrations. These sample concentrations and mass of non-volatile content were analyzed by two factor ANOVA with replication. See Table 2.18 and 2.19 for results.

Results.

Table 2.16. Mass of Non-volatile Extractable Content.

	non-volatile extractable content (mg)		
	bottom	middle	top
new growth	296	436	356
new growth	291	441	374
old growth	389	477	335
old growth	372	439	311
Average	337	448	344

Table 2.17. Sample PAH Content.

Sample Name	Concentrations of Analytes in Extracts (μg/L)					
	phenanthrene	anthracene	fluoranthene	pyrene	benzo(a) anthracene	chrysene
bottom new 1	18	1	44	32	7	446
bottom new 2	39	7	128	77	7	593
bottom old 1	29	6	61	64	6	43
bottom old 2	107	5	116	95	1	158
middle new 1	319	9	73	61	23	127
middle new 2	134	12	90	68	4	101
middle old 1	94	5	131	138	7	81
middle old 2	94	6	78	96	1	54
top new 1	37	0	25	13	0	249
top new 2	30	1	47	39	5	258
top old 1	60	3	83	52	3	74
top old 2	13	1	74	33	5	77

Table 2.18. ANOVA Table for Mass of Extracts.

Source of Variation	SS	df	MS	F-calc	P-value	F-crit
Needle Age	1387	1	1387	**6.20**	0.047	5.99
Location on Tree	31058	2	15529	**69.46**	7.1E-05	5.14
Interaction	8327	2	4163	**18.62**	0.0026	5.14
Within	1342	6	224			
Total	42113	11				

Table 2.19. ANOVA Tables for PAH Content.

ANOVA Table for Phenanthrene						
Source of Variation	SS	df	MS	F-calc	P-value	F-crit
Needle Age	2700	1	2700	0.75	0.419	5.99
Location on Tree	37876	2	18938	**5.28**	0.047	5.14
Interaction	16426	2	8213	2.29	0.182	5.14
Within	21504	6	3584			
Total	78506	11				
ANOVA Table for Anthracene						
Source of Variation	SS	df	MS	F-calc	P-value	F-crit
Needle Age	1	1	1	0.24	0.638	5.99
Location on Tree	96	2	48	**9.57**	0.014	5.14
Interaction	21	2	11	2.13	0.200	5.14
Within	30	6	5			
Total	149	11				

Table 2.19. (con't)

ANOVA Table for Fluoranthene						
Source of Variation	SS	df	MS	F-calc	P-value	F-crit
Needle Age	1513	1	1513	1.31	0.295	5.99
Location on Tree	2971	2	1485	1.29	0.342	5.14
Interaction	810	2	405	0.35	0.717	5.14
Within	6912	6	1152			
Total	12206	11				
ANOVA Table for Pyrene						
Source of Variation	SS	df	MS	F-calc	P-value	F-crit
Needle Age	2978	1	2978	**6.11**	0.048	5.99
Location on Tree	6535	2	3267	**6.71**	0.030	5.14
Interaction	692	2	346	0.71	0.529	5.14
Within	2923	6	487			
Total	13128	11				
ANOVA Table for Benzo(a)anthracene						
Source of Variation	SS	df	MS	F-calc	P-value	F-crit
Needle Age	47	1	47	1.22	0.311	5.99
Location on Tree	59	2	29	0.77	0.506	5.14
Interaction	60	2	30	0.78	0.500	5.14
Within	231	6	38			
Total	396	11				
ANOVA Table for Chrysene						
Source of Variation	SS	df	MS	F-calc	P-value	F-crit
Needle Age	137896	1	137896	**45.27**	0.001	5.99
Location on Tree	99253	2	49627	**16.29**	0.004	5.14
Interaction	71234	2	35617	**11.69**	0.009	5.14
Within	18278	6	3046			
Total	326661	11				

Bold indicates significant effects.

Discussion.

The goal of this experiment was to find out if samples of different age needles from different locations on the same tree would give insight into any non-homogenous characteristic of a tree as a sample source. From Table 2.18 it is clear that the non-volatile extractable content is not uniform throughout the tree. There is a difference in needle characteristic based on height location on tree. According to Table 2.16 the non-volatile extractable content of the needles is about 30% greater at middle height than at

the top and bottom of the tree. According to Table 4.19 four of the six PAH's the trees are heterogeneous. Phenanthrene, anthracene, fluoranthene, pyrene and benzo(a)anthracene concentrations are higher in the middle of the tree (Table 2.20).

Table 2.20. Average Concentrations at Each Height Location.

Location	Non-volatile extractable content (mg)	Average Concentrations at Height Locations (μg/L)					
		Phen-anthrene	Anthracene	Fluor-anthene	Pyrene	Benzo(a)-anthracene	Chrysene
Bottom	337	48	4.7	87	67	5.3	310
Middle	448	160	8	93	91	8.8	91
Top	344	35	1.3	57	34	3.3	165

Based on the non-volatile extractable content and PAH concentration differences within in a tree, samples collected from spruce trees for comparison to each other should all be taken from the same height.

2.5 Investigating Sources of Analyte Loss

Setup of Experiment

This experiment was set up to investigate the possible loss of analytes during the handling of the sample from extraction to final GC/MS analysis. The four areas that were investigated are loss to: extraction process, roto-evaporating to 1 mL, roto-evaporating to dryness and column chromatography. Table 2.21 shows the design of the experiment.

Table 2.21. Experimental Design for Percent Loss Experiment.

	Extraction	Roto-Evap to 1 mL	Roto-Evap to dryness	column chromatography	Loss Measured
Exp. #1 a & b*		X			roto-evaporation
Exp. #2 a & b			X		evaporating to dryness
Exp. #3 a & b					(control)
Exp. #4 a & b		X		X	roto-evaporation & column chromatography
Exp. #5 A	X		X	X	total loss
Exp. #5 B	X		X	X	(needle control)

* Expeiments 1 – 4 were performed in duplicate and are noted either a or b.

Spike Solution

A 1.00 mg/L PAH and 1.00 mg/L PAH internal standard solution was prepared by serial dilution of the 20 mg/L PAH stock solution and the 24.6 mg/L PAH internal standard stock solution.

Experiment 1 & 2.

For these experiments, 35 mL of hexane and 20 mL of 1:1 CH_2Cl_2:hexane was spiked with 1.00 mL of spike solution. This solution was then roto-evaporated to 1 mL. Two 0.25 mL aliquots were transferred to autosampler vials for experiments 1a and 1b. The remaining 0.5 mL was further evaporated to dryness under a steady stream of nitrogen and then reconstituted to 0.5 mL with hexane. This solution was split in half and transferred to autosampler vials for experiments 2a and 2b.

Experiment 3.

Experiment 3 is a control experiment. Two 0.5 mL aliquots of the spike solution were placed in autosampler vials and analyzed as experiments 3a and 3b.

Experiment 4.

A 1.00 mL aliquot of the spike solution was loaded on a prepared silica gel column. The column was eluted with 35 mL of hexane and then 20 mL 1:1 CH_2Cl_2:hexane. The eluate was collected as one fraction. The fraction was then roto-evaporated to about three milliliters and then transferred to a 3.0 mL conical vial. The fractions were then further concentrated by evaporating down to 1.0 mL under a steady stream of N_2. This solution was then split in half and transferred to GC/MS autosampler vials for experiments 4a and 4b.

Experiment 5.

Experiments 5A and 5B used 10 g of needles for Soxhlet extraction. Prior to extraction of 5A, 250 μL of spike solution was added to the needles in the glass thimble. Needles

were extracted for three hours with 125 mL of hexane. After the extractions were completed, the extracts were vacuum filtered through a fritted glass funnel and roto-evaporated to 1 mL. The concentrated extracts were then loaded onto prepared silica gel columns and eluted the same as in experiment 4. Originally the eluate were planned to be concentrated to 250μL. However, they were evaporated to an oily dryness and then reconstituted to 250 μL and transferred to GC/MS autosampler vials. These vials will be referred to as experiment 5A(spiked) and 5B (unspiked).

Analysis.

Each vial was analyzed for PAH content using the GC/MS method HOW3.M. A six-point calibration curve was made from 50, 125, 250, 500, 750, and 1000 μg/L PAH and internal standards. See Table 2.22 for results. Recoveries were calculated based on raw peak areas and also by using the internal standards. For experiments 1 through 4 the recoveries are averages of the duplicates analyses. For experiment 5, the concentrations in 5B were subtracted from the concentrations of 5B before calculating the recoveries.

Results

Table 2.22. Concentrations of Analytes and Internal Standard in Each Experiment.

	Concentration of analytes (μg/L)									
	Exp. 1a	Exp. 1b	Exp. 2a	Exp. 2b	Exp. 3a	Exp. 3b	Exp. 4a	Exp. 4b	Exp. 5A	Exp. 5B
acenaphthylene	733	712	121	129	891	1007	912	1192	856	27
acenaphthene	808	807	144	153	854	983	754	980	651	69
d10-acenaphthene	776	771	130	138	833	951	900	1170	916	0
fluorene	891	880	345	366	961	1012	970	1258	1091	126
phenanthrene	985	969	653	688	993	1026	1036	1316	1212	243
anthracene	1090	1061	726	760	1110	1062	1068	1372	1149	40
d10-phenanthrene	963	950	639	671	973	1016	1025	1288	1037	0
fluoranthene	935	918	781	823	921	999	1062	1318	1054	140
pyrene	926	903	789	827	921	992	1072	1339	974	105
benzo(a)anthracene	1494	1254	1117	1165	1164	1159	1408	1810	1575	50
chrysene	1422	1220	1094	1148	1140	1168	1422	1825	1630	87

Table 2.23. Recoveries Based on Raw Peak Areas.

Analyte	Recovery (%)				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
acenaphthylene	72	13	95	105	83
acenaphthene	81	15	92	87	58
d10-acenaphthene	77	13	89	104	92
fluorene	89	36	99	111	97
phenanthrene	98	67	101	118	97
anthracene	108	74	109	122	111
d10-phenanthrene	96	65	99	116	104
fluoranthene	93	80	96	119	91
pyrene	91	81	96	121	87
benzo(a)anthracene	137	114	116	161	152
chrysene	132	112	115	162	154

Table 2.24. Recoveries Using Internal Standard.

Analyte	Recovery (%)				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
acenaphthylene	94	94	107	102	91
acenaphthene	104	110	103	83	64
fluorene	92	54	98	95	94
phenanthrene	101	102	101	101	95
anthracene	112	113	109	105	107
fluoranthene	97	123	97	103	90
pyrene	95	123	96	104	85
benzo(a)anthracene	148	180	121	144	152
chrysene	142	177	120	145	154

Discussion

The largest source of analyte loss is from roto-evaporating to dryness (Table 2.23). Allowing the extract to evaporate to dryness should be avoided. The use of internal standard partially corrects the problem (Table 2.24). Because of the wide range in volatility of the analytes, multiple internal standards are required. D10-acenaphthene is an excellent internal standard for acenaphthylene and acenaphthene. D10-phenanthrene is an appropriate internal standard for phenanthrene, anthracene, fluoranthene and pyrene. The recoveries of benzo(a)anthracene and chrysene are included in the above tables

however their results seem extraordinarily high. The GC/MS SIM program was not edited to include the internal standard, d12-chrysene, for these analytes. This would explain the slightly high values for the internal standard corrected recoveries in Experiment 3, which were based on d10-phenanthrene. In section 2.2.4 recoveries for these to analytes ranged from 103 to 128 % using the d12-chrysene internal standard. This suggests that using the appropriate internal standard the for benzo(a)anthracene and chrysene produces acceptable recoveries.

2.6. Conclusion of Method Development.

The overall goal of this method development was to investigate the steps involved in preparing spruce needle extract for GC/MS analysis. A primary factor was to streamline conventional procedure without jeopardizing the quantitation of the analytes. In parallel to the experiments described here, Shane Billings simultaneously investigated the effect of the above method development on the analysis of pesticide content, notably hexachlorobenzene (20).

Elution profile experiments showed that simplifying the elution scheme to a single solvent, 1:1 CH_2Cl_2:hexane, reduced the work necessary to do adequate clean-up chromatography. An investigation into the sources of analyte loss during the sample preparation showed that evaporating to dryness before and /or after clean-up chromatography is the most significant source of analyte loss.

The investigation into the extraction process also gave insight into simplifying the extraction procedure. All variables manipulated in the extraction investigation showed no significant effect on the determination of PAH content of spruce needles. Grinding the needles prior to extraction, however, was a very important variable in the determination of non-volatile extractable content. Grinding the needles dramatically increased the non-volatile extractable content. Based on these results, the most efficient extraction procedure is to soak 25 g of ground needles in 75 mL of hexane for two hours

without heating or agitation. This approach would greatly reduce the time, materials and monitoring necessary for successful extraction of PAHs from spruce needles.

Unfortunately, this process was not adopted completely. The same investigation for pesticides by Shane Billings indicated that the amount of pesticide extracted is much greater with Soxhlet extraction than with soaking (20). Since the same extracts were analyzed for both PAH and pesticide content, a two hour Soxhlet extraction with 75 mL of hexane was used in this work.

An evaluation of the analytical chemistry throughout the method development shows a few things. One, using single point regression is well justified. By looking at results of calibration curves we see that the linear behavior in the 50 to 1000 μg/L range is very consistent (Figure 2.8).

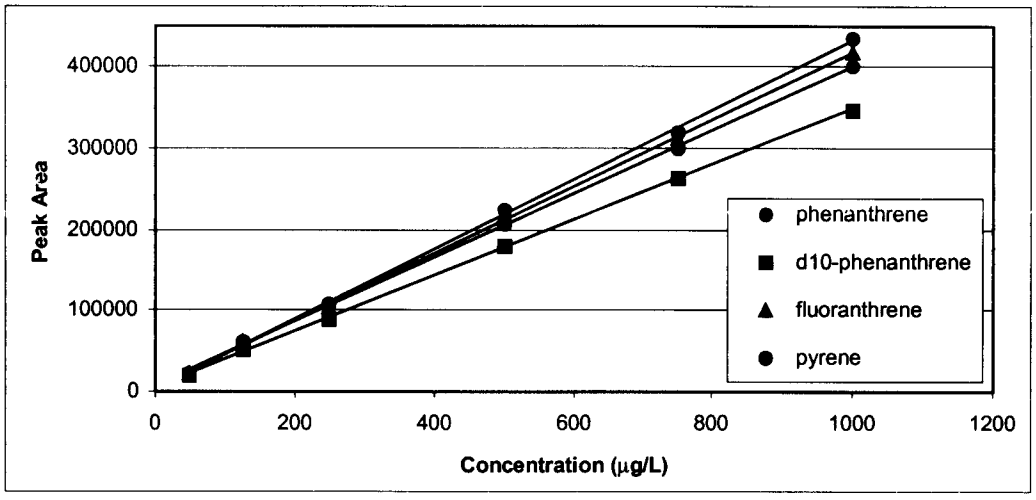


Figure 2.8. Typical calibration curve based on least square linear regression.

The instrument detection limit was also estimated in Table 2.4. For all but benzo(a)anthracene and chrysene the instrument MDL was about 7 μg/L. Benzo(a)anthracene and chrysene had MDLs about twice that value. According to Table 2.7, GC/MS analysis for all but benzo(a)anthracene and chrysene had an RSD of about

6%. The RSD for benzo(a)anthracene and chrysene was about 17%. The GC/MS method described in Table 2.2 is not ideal for benzo(a)anthracene and chrysene analysis. Recovery of benzo(a)anthracene and chrysene throughout method development also showed inconsistencies with the other analytes. For these reason benzo(a)anthracene and chrysene will not be considered in the analysis of environmental samples in the subsequent chapters. Flourene will also be excluded from further analysis, due to lack of an appropriate internal standard.

3.0 Environmental Sample Collection, Preparation and Analysis.

3.1 Sample Collection.

Spruce needle samples were collected over four days in early April 1997. Samples were collected near major roadways throughout Alaska. The goal was to travel from Fairbanks to other parts of the state and collect samples at approximately 50 mile intervals with exception of much smaller interval to compensate for dramatically changing terrain (e.g. the first 100 miles of the Richardson highway). At each sample location, Spruce branches were collected from three to five locations on two tree at approximately 1.5 m. from the ground. The branches were then chopped with an ax into about six inch pieces and placed into a one-gallon Ziploc bag. Each bag was filled to capacity with pieces picked randomly from a larger pile of chopped branches. The bags were sealed and placed in a cooled insulated box. Sample location, species of spruce, sampling date and tree number were recorded on each bag. Tree number distinguishes duplicate samples as either tree #1 or #2 from each site. Geographical and ecological descriptions of each location are given in Table 3.1 and Appendix A.

Table 3.1. Description of Sampling Locations.

Highway	Milepost	Species	Latitude* (degrees)	Longitude* (degrees)	Elevation* (feet)	Ecosystem*
Rich	4	Sitka	61.10	146.21	200	c
Rich	16	Sitka	61.09	145.87	400	c
Rich	31	Missing	61.18	145.63	2000	a
Rich	72	Black	61.60	145.21	1750	u
Rich	109	White	62.08	145.37	1420	u
Rich	166	Black	62.82	145.49	2800	u
Rich	207	Missing	63.33	145.71	2500	mt
Rich	277	White	64.23	146.00	1000	mb
Rich	309	White	64.37	146.84	800	mb
Rich	339	Black	64.72	147.22	500	l
Dalton	13	White	65.58	148.99	1100	mb
Dalton	50	Black	65.85	149.68	1100	u
Dalton	87	Black	66.27	150.29	1500	u
Dalton	122	Black	66.67	150.63	850	mt

Table 3.1. (Con't)

Highway	Milepost	Species	Latitude*	Longitude*	Elevation*	Ecosystem*
			(degrees)	(degrees)	(feet)	
Dalton	160	Black	67.14	150.29	900	u
Dalton	193	Black	67.54	149.78	1500	u
Elliot	9	Black	65.09	147.58	600	mb
Elliot	47	White	65.36	148.24	800	u
Parks	67	White	62.16	150.12	400	l
Parks	107	White	62.69	150.22	800	u
Parks	147	White	63.12	149.44	1800	u
Parks	187	Black	63.54	148.78	2000	u
Parks	214	White	63.89	149.03	1400	u
Parks	230	White	64.10	149.21	1000	u
Parks	270	Black	64.59	149.10	300	b
Seward	8	Sitka	60.14	149.38	200	c
Seward	42	Sitka	60.65	149.51	1400	c
Seward	75	Sitka	60.83	148.99	0	c
AK	1230	Black	62.68	141.09	2100	mb
AK	1260	Black	62.90	141.53	1900	u
AK	1301	Black	63.26	142.41	1800	b
AK	1345	White	63.39	143.79	1500	u
AK	1384	White	63.70	144.62	1300	u
AK	1418	Black	63.97	145.50	1200	l
Goldstream		White	64.86	147.88	482	l

* Data obtained from USGS maps

KEY for ecosystem description.

c	coastal western hemlock-sitka spruce forest
mt	moist tundra
u	upland spruce hardwood forest
l	lowland spruce hardwood forest
mb	muskeg-bog
a	alpine tundra
b	bottomland spruce forest

Samples were preserved by storing the bags in a chest freezer at –20 °F. Each bag was taken out the freezer for about 30 to 45 min so that the needles could be clipped from the branches and returned to the freezer. The contents of each bag were placed on a clean table and the needles were clipped from the pieces of branches with handheld grass

clippers. The clipped needles were then placed back into their original bags and placed back into the freezer until extraction time.

3.2 Sample Preparation

3.2.1 Extraction Replication and Spiking Scheme

Two different trees were sampled at each site. Table 3.2 also indicates which tree samples were extracted in duplicate and which tree samples were also spiked prior to extraction.

Table 3.2. List of Sample Locations

Highway	Milepost	Highway	Milepost	Highway	Milepost
Richardson	4	Parks	67	Alaska	1230*
	16 *		107*		1260
	31**		147**		1301**
	72		187		1345
	109		214		1384
	166*		230*		1418*
	207**		270**		
	239			Dalton	13**
	277	Seward	8**		50*
	309*		45*		87
	339**		75**		122
					160**
Elliot	9**				193*
	47*				

* Indicates that both tree samples were extracted twice, once with spike and once without.

** Indicates that both tree sample were extracted in duplicate.

3.2.2 Preparation of Sample Extractions

3.2.2.1 Glassware Treatment

All glassware was washed with hot soapy water and rinsed numerous times with deionized water. Glassware was baked at 250 °C overnight.

3.2.2.2 Extraction

Soxhlet apparatus was used to extract the samples. The needles were ground in an electric coffee bean grinder (DeLonghi, #DCG-1) until the finely ground light illuminated. Ten grams of the ground needles were placed inside a glass Soxhlet thimble. The thimble was then slid into the Soxhlet apparatus, which was then connected to a 250 mL round bottom flask filled with 125 mL of hexane and five glass boiling beads. A vertical condenser was connected to the top of the apparatus. Six condensers were connected in series with a circulating cryogenic pump filled with 30/70 water/antifreeze. Prior to start of extraction, the antifreeze was chilled to -35 °C. Six Soxhlet extractions were performed in parallel. Heat was applied to the round bottom flask using electric heating mantles set at low settings. Once thimbles started to fill with solvent, the extraction was continued for three hours with a thimble fill and drain time of 2 min.

3.2.2.3 Quality Control Measures

Samples were prepared and extracted in a randomized order. Prior to placing the thimbles into the apparatus, 250 μL of 250 μg/L internal standard was directly added to the top of the filled thimble using a 500 μL gas-tight syringe. Samples to be spiked also received a 250μL addition of 250 μg/L PAH standard. Each batch of extractions also had a method blank. The method blank consisted of an empty thimble with 250 μL of 250 μg/L internal standard added to it. Method blanks were extracted and prepared in the same manner as the samples. The internal standard was also used as the surrogate standard for each extraction.

3.2.2.4 Clean-up Chromatography and Concentration of Extracts.

After the extraction was completed, the extracts were gravity filtered through a fritted glass funnel and returned to the 125 mL round bottom flask. The funnel and filtration vessel were then rinsed three times with 15 mL of hexane. The rinsings were added to the extract. The extract was then roto-evaporated to about 3 mL and transferred to a

conical vial. Prior to clean-up chromatography the extracts were further evaporated to approximately 1 mL under a steady flow of N_2. The conditions for clean-up chromatography are in Table 3.3. Pressure was applied to the top of column using compressed N_2 gas.

Table 3.3. Clean-up Chromatography Conditions.

Glass Column	45 cm x 1.0 cm
Stationary Phase	4.0 g silica gel
Mobile Phase	20 mL 1:1 CH_2Cl_2:Hexane
Flow Rate	1 – 1.5 mL /min.

The eluate was collected in 50 mL round bottom flask and concentrated to approximately 0.3 mL in the same manner as prior to clean-up chromatography. The volume of concentrated eluate was measured using a 500 μL gas-tight syringe. The eluate was transferred to a GC/MS autosampler vial for analysis.

3.2.3 Determination of Water Content and Non-volatile Extractable Content

Water content of the needles was determined by placing 10 g of needles in a drying oven set at 55 °C for 24 hrs. The difference between the mass of needles before and after drying was considered the water content of the needles.

Non-volatile extractable content was determined by placing a 3.00 mL aliquot of the filtered extract into a tared vial and letting the solvent evaporate at room temperature. The mass remaining in the vial after all the solvent had evaporated was considered the non-volatile extractable content of the needles.

3.3 GC/MS Analysis

All samples were analyzed using GC/MS method HOW3NEW.M. Samples were analyzed in 13 sequence batches. For each sequence batch, samples were chosen at random from the available prepared sample extracts. Each sequence batch followed the same series of events described in Table 3.4.

Table 3.4. Sequence Batch Event Order.

Sequence Order	Event
1	Autotune
2	Column Bake
3	Standard 1a
4	Instrument Blank 1a
5-9	5 samples
10	Standard 1b
11	Instrument Blank 1b
12	Column Bake
13	Standard 2a
14	Instrument Blank 2a
15-19	5 samples
20	Standard 2b
21	Instrument Blank 2b

Column Bake refers to a GC/MS method that thoroughly rinses the syringe and then conditions the column at 280°C for 2.5 hrs. Analytes were quantified using an average single point calibration based on a 250 μg/L standard. For example, samples from sequence order 5 through 9 were quantified using the average analyte/internal standard value of standard 1a and 1b. Instrument blanks were used to verify the lack of carry over from preceding standards. The validity of using a single point regression is well defined throughout the Method Development chapter. Analyte concentrations for fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo (a)anthracene and chrysene were reported as nanogram/gram dry mass of needles.

3.4 Data Analysis

All GC/MS results were screened using quality control criteria. Removal of potentially erroneous data was determined based on internal standard recovery limit of 60 to 140%. Method blanks were analyzed to determine contamination during sample preparation. Instrument blanks were analyzed to confirm the lack of carry over from the autosampler. Duplicate samples were analyzed to estimate reproducibility. Sample spikes and internal standards were analyzed to estimate extraction efficiency.

The average site values were analyzed using The Unscrambler 6.11a software(CAMO ASA. Oslo, Norway.) This software allowed for a multivariate statistical analysis of the PAH concentrations and non-volatile extractable content along with the site and sample description variables. Species, latitude, longitude, elevation and ecosystem data are included in Table 3.1. Additional variable data are listed in Table 3.5.

Table 3.5. Additional Site and Sample Description Data.

Sample highway	Location milepost	Radial distance* from urban site (miles)	Urban site	Temperature** (°F)	Precipitation** (inches)
AK	1230	missing	Fairbanks	-6.0	missing
AK	1260	missing	Fairbanks	-5.0	0.85
AK	1301	missing	Fairbanks	-4.9	0.75
AK	1345	157	Fairbanks	-3.1	0.99
AK	1384	125	Fairbanks	1.1	0.9
AK	1418	92.5	Fairbanks	4.8	0.86
Dalton	122	157	Fairbanks	-2.5	0.97
Dalton	13	62.5	Fairbanks	-1.1	0.76
Dalton	160	177	Fairbanks	-2.6	1.08
Dalton	193	200	Fairbanks	-2.4	1.1
Dalton	50	92.5	Fairbanks	-1.6	0.84
Dalton	87	125	Fairbanks	-2.2	0.9
Elliot	47	42.5	Fairbanks	-0.7	0.74
Elliot	9	17.5	Fairbanks	0.0	0.65
Goldstream		2.5	Fairbanks	0.5	0.7
Parks	107	105	Anchorage	16.8	1.68
Parks	147	135	Anchorage	13.1	1.08

Table 3.5. (con't)

Sample highway	Location milepost	Radial distance* from urban site (miles)	Urban site	Temperature** (°F)	Precipitation** (inches)
Parks	230	70.0	Fairbanks	4.0	0.42
Parks	270	45.0	Fairbanks	0.8	0.4
Parks	67	62.5	Anchorage	20.3	2.36
Richardson	109	207.5	Fairbanks	-3.7	1.26
Richardson	16	13.8	Valdez	27.8	14.64
Richardson	166	155	Fairbanks	5.7	1.5
Richardson	207	122.5	Fairbanks	6.6	1.5
Richardson	277	67.5	Fairbanks	5.4	0.9
Richardson	309	42.5	Fairbanks	3.9	1
Richardson	31	22.5	Valdez	missing	20
Richardson	339	13.8	Fairbanks	1.3	0.58
Richardson	4	4.5	Valdez	27.8	14.64
Richardson	72	50.0	Valdez	5.0	1.31
Seward	42	42.5	Anchorage	23.1	7.5
Seward	75	40.0	Anchorage	22.6	5
Seward	8	75.0	Anchorage	31.7	13.63

* Data obtained from USGS maps

* * Data was obtained from the Western Regional Climate Center (URL:www.wrcc.sage.dri.edu). Temperature data is based on three-month average of January through March 1997. Precipitation data is sum of both snow and rain accumulation during the months of January through March 1997, expressed as wet precipitation equivalence.

The categorical variables, ecosystem and species, were coded as matrices of +1 or -1. For example, the species variable would be changed into a matrix of three variables: sitka, black and white. Each variable would receive a +1 or –1 based on the species designation for that sample. A Sitka spruce sample would be coded as Sitka = +1, Black = -1, White = -1.

Forest fire information was also included as part of the data set. Each sample site was ranked on a scale of 0 to 3 for potential impact from forest fires. Potential forest fire impact was estimated from Figure 3.1.

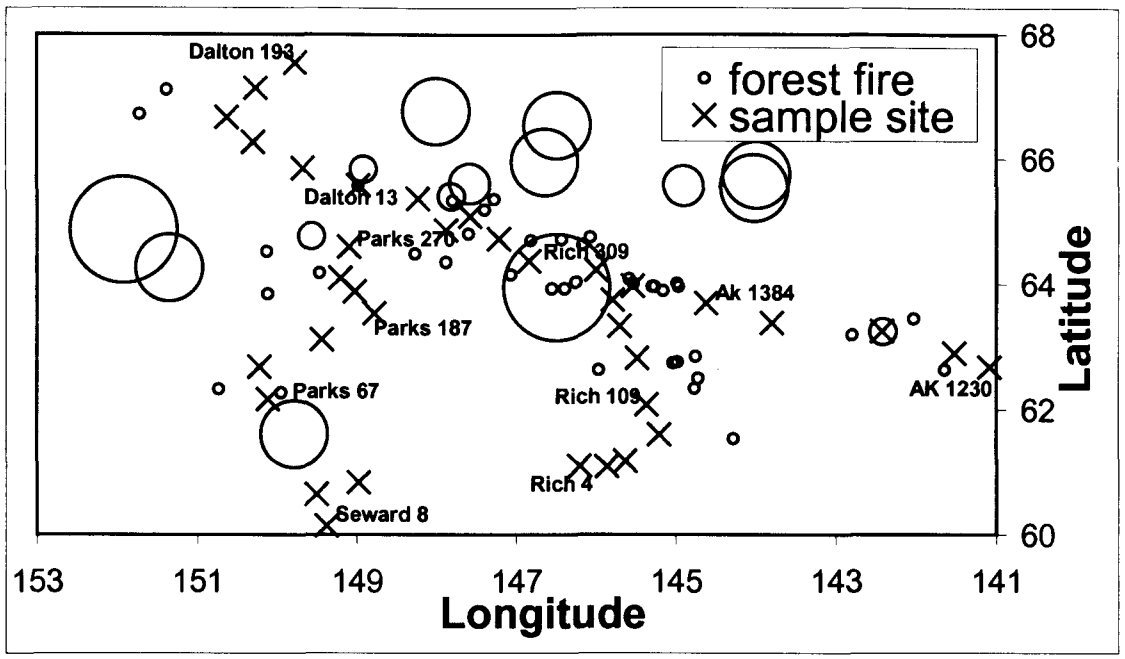


Figure 3.1. Plot of 1996 and 1997 forest fire and sample site locations. Size of forest fire circle reflects magnitude of fire. The largest circles mark fires greater than 50,000 acres. The smallest circles mark fires smaller than 2,000 acres. Fires less than 10 acres were excluded. Forest fire information was obtained from Bureau of Land Management.

Sample sites within the large forest fire circles, i.e. Rich 309, were given a forest fire impact rank of 3. Sample sites relatively far from forest fire, for example Rich 4 and Dalton 193, were given ranks of 0. Sample sites like Parks 270, which are surrounded by fires but not very close to large fires were given a rank of 2. Samples like AK 1384 were given a rank of 1 because they although they as far from any major fires they are relatively close to small fires. Appendix B has the list of the forest fire locations, dates and size of fires used to construct Figure 3.1. Table 3.6 is a list of each sample site's potential forest fire impact rankings.

Table 3.6. List of Sample Sites and Potential Forest Fire Impact Rankings.

Sample site	Fire impact rank	Sample site	Fire impact rank	Sample site	Fire impact rank	Sample site	Fire impact rank
AK 1230	2	Dalton 193	1	Parks 187	1	Rich 166	2
AK 1260	2	Dalton 160	1	Parks 214	2	Rich 207	2
AK 1301	3	Dalton 122	1	Parks 230	2	Rich 277	3
AK 1345	1	Elliot 9	2	Parks 270	2	Rich 309	3
AK 1384	2	Elliot 47	2	Rich 4	0	Rich 339	2
AK 1418	3	Goldstream	2	Rich 16	0	Seward 8	0
Dalton 13	2	Parks 67	2	Rich 31	0	Seward 45	1
Dalton 50	1	Parks 107	2	Rich 72	1	Seward 75	1
Dalton 87	1	Parks 147	1	Rich 109	1	Dalton 122	1

4.0 Results and Discussion

4.1 Analysis of Chromatograms.

All chromatograms were integrated using the integrator provided by Hewlett Packard Chemstation Version C.03.00. A typical SIM chromatogram is shown in Figure 4.1. The molecular ion m/z value for each analyte was extracted from the original chromatogram to produce its own extracted ion chromatogram (EIC). The EICs were then integrated separately (See Figure 4.2).

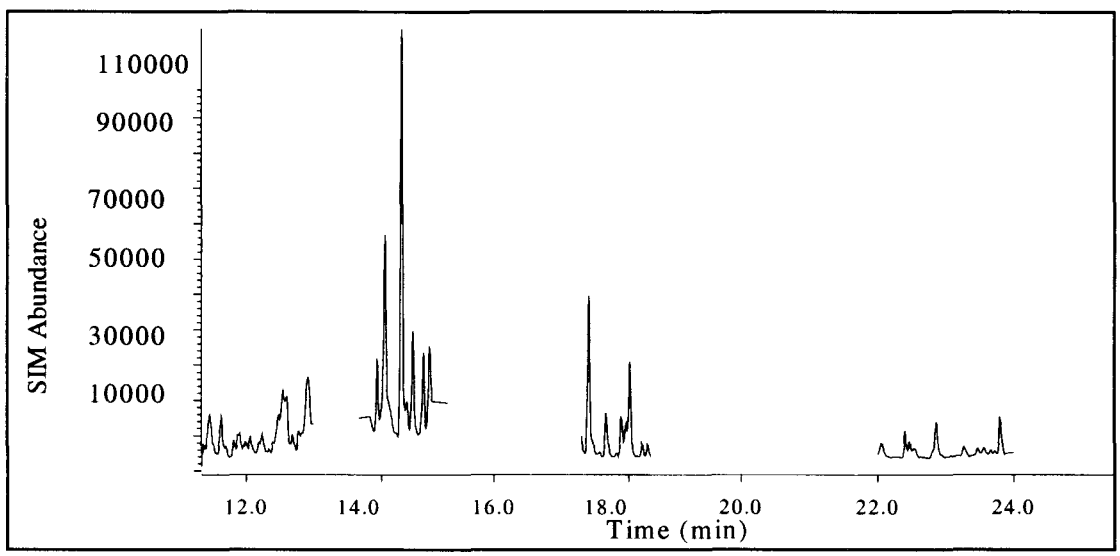


Figure 4.1 SIM Chromatogram for sample Parks 214 tree 2. Blank portions are times when the detector was turned off.

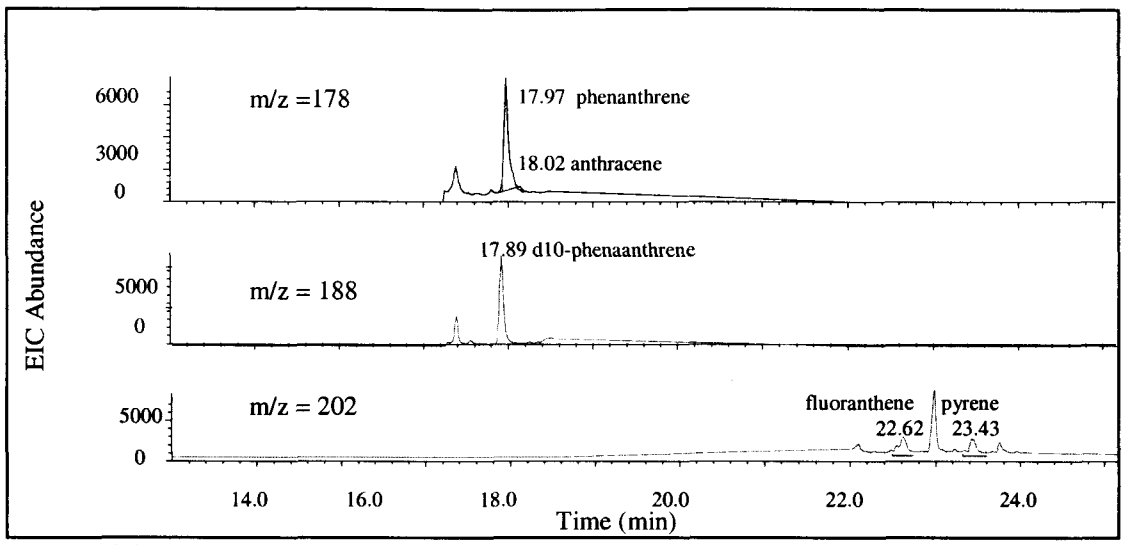


Figure 4.2 Extracted ion chromatogram for Sample Parks 214 tree 2. Analyte identities and retention times are shown.

An analyte peak confirmation was done by comparison of target ion ratio and retention time to standard chromatograms (refer to Table 2.3.). The section of chromatogram, between 11 and 13 minutes, containing acenaphthylene, acenaphthene, and d10-acenaphthene was not interpretable and these analytes could not be quantified. The analytes that were quantified reliably were phenanthrene, anthracene, fluoranthene, and pyrene. Analytes were quantified using internal standard, d10-phenanthrene. A complete list of chromatographic results can be found in Appendix C.

4.2 Quality Control Analysis

4.2.1 Method Blanks

Method blanks were not free of analytes. The blank concentrations generally decreased over time. A least square linear regression of blank levels and extraction date was used to correct samples for contamination. Figure 4.3 shows an example of the trend in decreasing blank levels with time. Table 4.1 lists the coefficients of the equations used to correct the sample data.

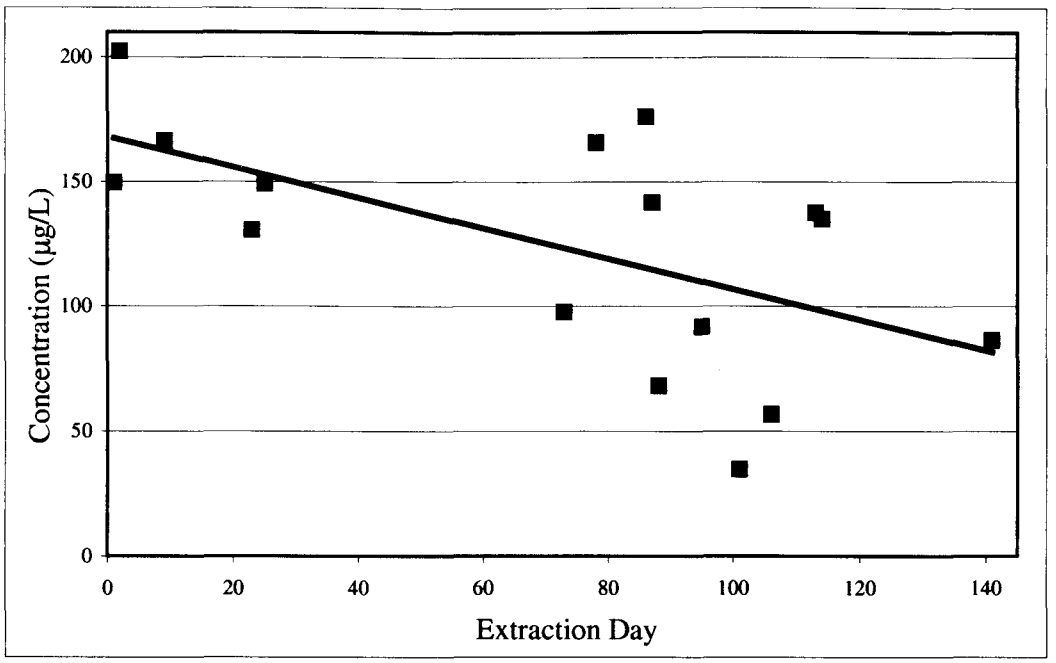


Figure 4.3. Plot of phenanthrene method blank concentration versus time, including linear trendline.

Table 4.1. Blank Correction Equation Slopes and Intercepts.

Analyte	Slope ((µg/L)/day)	y-intercept (µg/L)
Phenanthrene	-0.612	168
Anthracene	-0.047	7.5
Pyrene	-0.190	40.1
Fluoranthene	-0.104	29.0

For all analytes the slope of correction equation had a negative value indicating a decrease in method blank level over the course of the extraction sequence. The blank corrected concentrations were calculated by subtracting the method blank concentration from the sample concentration. The method blank concentration was calculated using the slopes and y-intercepts from Table 4.1.

4.2.2 Surrogate Recovery

Figure 4.4 shows the distribution of recovery of the surrogate, d10-phenanthrene.

The average recovery of the surrogate was about 80%. Over 85% of the samples fell within a range of 60 – 140%. A complete list of surrogate recoveries can be found in Appendix C, Table C-4.

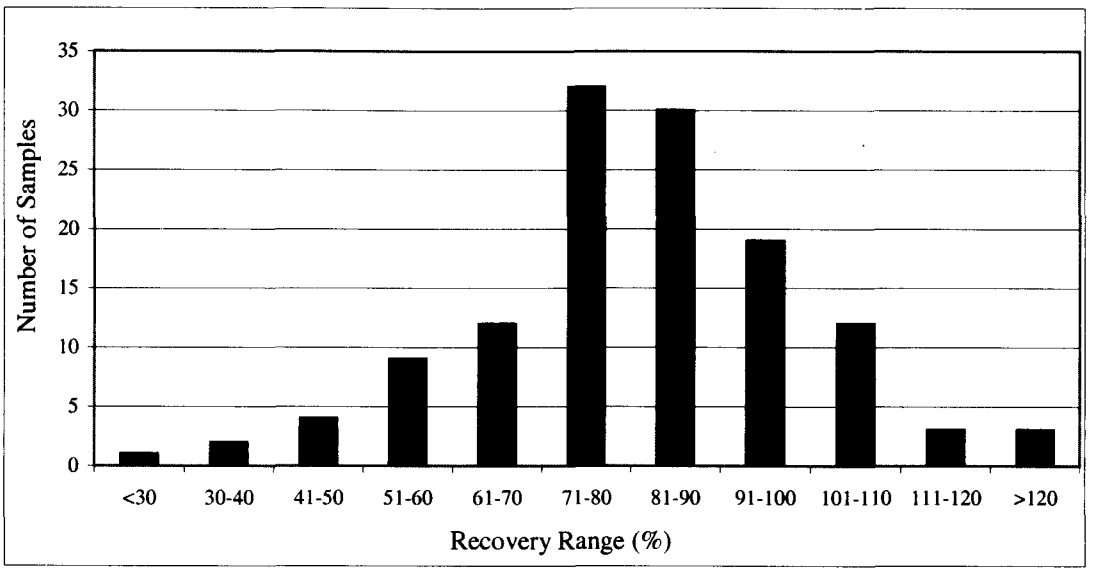


Figure 4.4. Distribution of d-10 phenanthrene recovery.

4.2.3 Spike Recoveries

Average recoveries of phenanthrene, fluoranthene, and pyrene from spiked samples were greater than 90% (Table 4.2). Refer to Appendix C, Table A-4, for the concentrations of analytes in spiked and unspiked samples.

Table 4.2. Analyte Recoveries for Spiked Samples.

Sample Name	Recovery of Analyte (%)			
(hwy milepost.tree #)	Phenanthrene	Anthracene	Fluoranthene	Pyrene
Ak 1230.1	110	102	105	93
Ak 1230.2	97	96	87	91
Ak 1418.1	81	97	93	96
Ak 1418.2	80	98	89	91
Dalton 193.1	86	91	89	97
Dalton 193.2	112	96	87	94
Dalton 50.1	103	87	80	88
Dalton 50.2	102	103	77	91
Elliot 47.1	136	94	97	90
Elliot 47.2	-3*	103	72	71
Parks 107.1	135	99	94	119
Parks107.2	152*	101	114	100
Parks 230.1	112	98	89	106
Parks 230.2	87	97	106	101
Rich 16.1	113	100	95	81
Rich 16.2	62	98	100	95
Rich 166.1	87	100	85	88
Rich 166.2	115	103	94	107
Rich 309.1	182*	165*	169*	163*
Rich 309.2	89	100	102	80
Seward 42.1	77	93	83	84
Seward 42.2	76	95	92	84
Average Recovery	98	98	92	93

* indicates recoveries outside of 60-140%

Almost all spiked samples had recoveries within the 60 - 140% range. A notable exception is sample Rich 309.1, which seemed to have a spike recovery of about twice what was expected. This could have been from the sample being spiked twice during preparation. Elliot 47.2 also had suspect recoveries, however both the spiked and unspiked samples had surrogate recoveries well below 60%.

4.2.4 Duplicate Samples and Extractions

Estimates of standard deviation were made from paired data, using the equation :

$$Sp = \sqrt{\frac{\sum d^2}{2k}}$$

The pooled standard deviation, Sp, from two extractions from the same tree gives an estimate of analytical variance (Table 4.3). The pooled standard deviation from two trees from the same location gives an estimate of the sum of analytical variance plus biological variance (Table 4.4).

Table 4.3. Duplicate Extraction Results (2 extractions from one tree).

duplicate extractions; k=17.	average concentrations	within tree pooled standard deviations
	(ng/gram dry needles)	
phenanthrene	28	10
anthracene	1.4	0.5
fluoranthene	10	3
pyrene	9	4
non-volatile extractable content (mg/10 g needles)	175	15

Table 4.4. Duplicate Tree Results. (tree #1 vs. tree #2).

duplicate samples; k=21	average concentrations	total pooled standard deviations
	(ng/gram dry needles)	
phenanthrene	23	13
anthracene	0.9	0.5
fluoranthene	7	3
pyrene	8	5
non-volatile extractable content (mg/10 g needles)	192	31

Table 4.3 and 4.4 show that PAH concentration within tree variability is approximately equal to the total variability. This indicates that analytical variability predominates in the PAH concentration measurements. The opposite is true with the non-volatile extractable content. The tree to tree variance (estimated using relationship: (tree to tree)^2 +

$(\text{extraction})^2 = (\text{total})^2$) is about 90% of the total variance in the non-volatile extractable content. This indicates that biological variability predominates in the non-volatile extractable content measurements.

Although biological variability accounts for almost all non-volatile extractable content measurement variability it is not apparent in the PAH concentration measurements. This is not surprising since the analytical procedure for PAH measurement is extensive. It is therefore reasonable to expect the analytical variability of PAH concentration measurements to be much greater than the biological variability.

4.2.5 Summary of Quality Control Analysis.

The quality control analysis indicates that, in general, the results are acceptable. The blank values were non-constant, but small. Blank correction was applied. Interpretation of the surrogate recoveries indicates that the extraction and GC/MS analysis were acceptable for phenanthrene, anthracene, fluoranthene and pyrene. An evaluation of the duplication results indicates the need to average both tree sample concentrations at each site before any geographical interpretation of the results is performed.

4.3 Analytical Results

Table 4.5 lists the average concentration of analytes and non-volatile extractable content at each site. Refer to Appendix D for an explanation of how the concentration calculations were made.

Table 4.5. Average Site Non-volatile Extractable Content and Analyte Concentrations.

Sample Name (highway milepost)	Non-volatile extractable content (mg/10 g needle)	Concentrations (ng/g dry needle mass)			
		phenanthrene	anthracene	fluoranthene	pyrene
Ak 1230	162	8	0.2	1.6	4.4
Ak 1260	530	60	9.7	18	10
Ak 1301	108	35	2.1	10	8.4
Ak 1345	89	18	0.7	7.7	13
Ak 1384	92	8	0.5	4.0	4.7
Ak 1418	165	33	1.3	9.3	7.8
Dalton 122	335	18	0.8	1.0	8.9
Dalton 13	156	23	0.8	5.0	2.3
Dalton 160	219	30	1.4	8.8	8.4
Dalton 193	139	12	1.2	4.8	3.8
Dalton 50	357	24	0.9	4.9	4.3
Dalton 87	167	34	1.0	8.0	17
Elliot 47	333	27	0.4	5.7	4.4
Elliot 9	136	23	1.3	10	8.2
Goldstream	168	57	1.3	44	39
Parks 107	170	24	0.6	7.1	5.9
Parks 147	99	28	1.6	8.6	10
Parks 187	106	20	0.5	6.8	8.7
Parks 214	147	13	0.6	3.8	6.6
Parks 230	157	25	0.6	9.5	9.3
Parks 270	388	48	1.3	11	14
Parks 67	97	36	2.9	18	16
Rich 109	154	43	3.7	9.8	3.8
Rich 16	174	31	1.4	6.6	5.3
Rich 166	237	21	0.4	3.4	8.7
Rich 207	128	29	1.3	14	14
Rich 277	126	15	0.7	9.5	15
Rich 309	141	25	0.8	9.3	15
Rich 31	108	13	1.1	5.8	6.5
Rich 339	231	89	1.8	53	36
Rich 4	208	39	0.4	8.9	6.3
Rich 72	190	19	0.5	4.0	4.3
Seward 42	173	25	1.9	8.7	6.7
Seward 75	80	20	2.6	9.6	5.5
Seward 8	211	42	3.3	17	16
Range of values	80-530	8-89	0.2-3.7	1.0-53	2.3-39

Samples that had surrogate recoveries outside the range of 60-140 % were excluded from the averages. Table 4.6 lists the samples excluded.

Table 4.6. Excluded Data.

Sample Name
Ak 1230 tree 2
Ak 1301 tree1, duplicate a
Ak 1301 tree1, duplicate b
Dal 122 tree2
Dal 87 tree 2
Goldstream tree 1
Parks 214 tree 2
Parks 270 tree 1, duplicate a
Rich 16 tree 1
Rich 4 tree 1

The concentration reported in Table 4.5 are in the low end of the range of concentrations found by other research groups that have measured PAH concentrations in conifer needles. Previous researchers have reported phenanthrene, anthracene, fluoranthene, and pyrene needle concentrations in the ranges of 6- 1800, 0.3 –22, 3-458, and 3-233 ng/g dry needle, respectively (6,11,21).

4.4 Multivariate Analysis of Data.

The ultimate goal of the analysis of the data is to investigate the relationship between PAH concentration in spruce needles and climate and geographical variables. A partial least squares multivariate regression (PLS) model was developed to investigate these relationships. PLS models both the X- and Y-matrices simultaneously to find which latent variables in X, in this case climate and geographical variables, best describe the latent variable in Y, analyte concentration.

PLS models were developed for each of the four analytes using the parameters listed in Appendix E.

4.5 Optimization of Statistical Analysis

Before a model is made and interpreted, outliers have to be evaluated and a decision on the optimal number of PCs must be made. An evaluation of outliers was based on the relative magnitude of its residual to the model. Data that contained extreme residuals in the principle components of interest were removed from the model. Outliers were evaluated and removed on a model by model basis (See Table 4.7).

Table 4.7. List of Outlier Data.

Analyte Model	Outlier Sample Sites (hwy. milepost)
Phenanthrene	Ak 1260, Goldstream, Richardson 109 & 339
Anthracene	Ak 1260 & 1301, Richardson 4 ,16, & 109
Fluoranthene	Ak 1260, Goldstream, Richardson 207 & 339, Seward 8
Pyrene	none

Determination of the optimum number of principle components was accomplished by evaluating the total residual Y-variance. This is a measure of the error made when the observed sample or variable is replaced by its projection onto the model. It can be viewed as an expression of the modeling or prediction error. Figure 4.5 shows the total residual Y-variance for the models as a function of the number of PC's chosen.

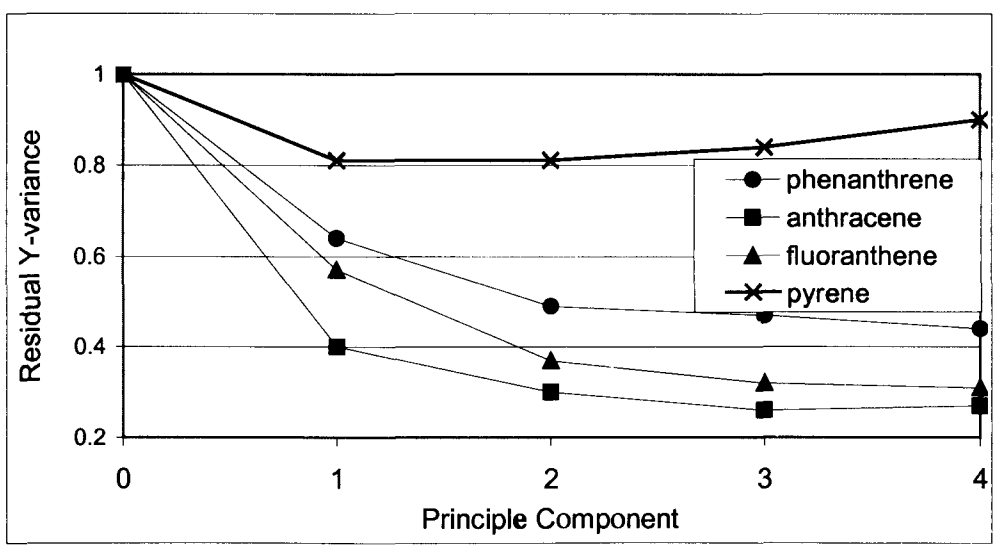


Figure 4.5. Total residual Y-variance plot for PLS-1 models.

Typically the residual Y-variance decreases with the addition of more PCs. The variance can reach a minimum value and then start to increase, as a result of overfitting the data, as is the case with pyrene. For the other three models, residual Y-variance sharply decreases with the addition of the first two PCs and changes little with the addition of more PCs. Therefore, choosing the first two PCs to describe correlation among variables is justified. The exception would be the pyrene model, which can be interpreted using only the first PC.

4.6 Interpretation of Statistical Analysis

An investigation of variables' relationship to each other is accomplished by evaluating a scatter plot of X-loading weights and Y-loadings for the two most important principle components from the PLS models. This type of plot shows the importance of the different variables for the two PC's selected. It can thus detect important variables and show the relationships between X- and Y-variables. For the four analyte models, a plot of PC-1 versus PC-2 is most useful since these two PC's account for the majority of variation in Y, analyte concentration (Figure 4.6 - 9).

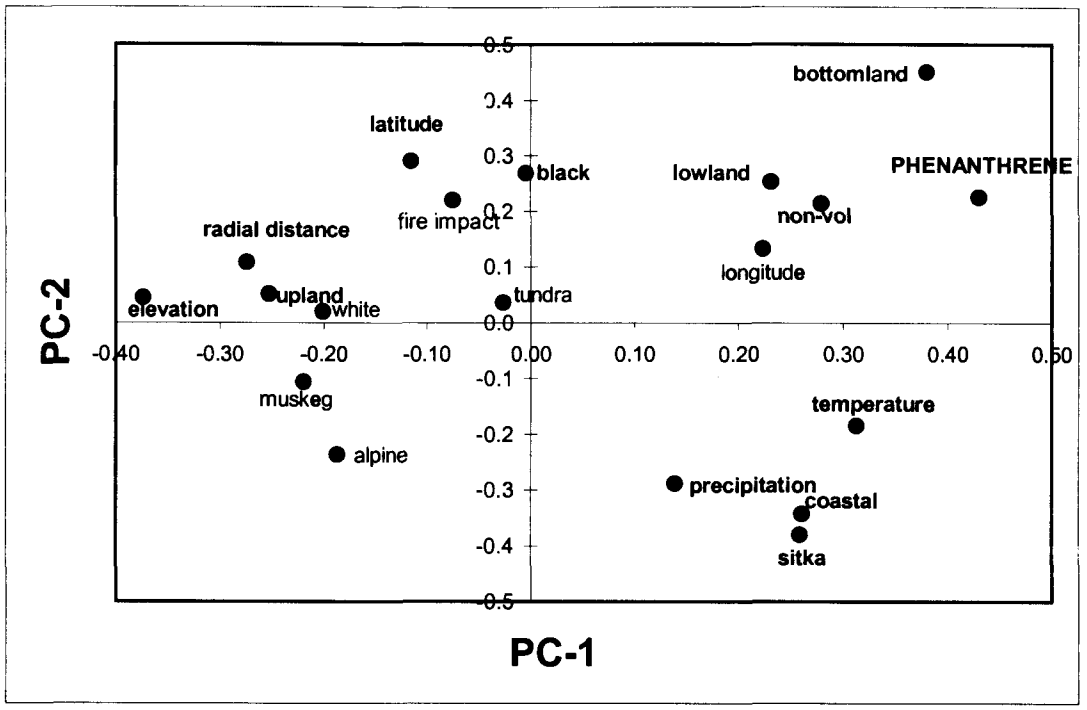


Figure 4.6. X loading weights and Y loadings for phenanthrene PLS-1 model. X explained 19%, 16%. Y-explained 42%, 14%.

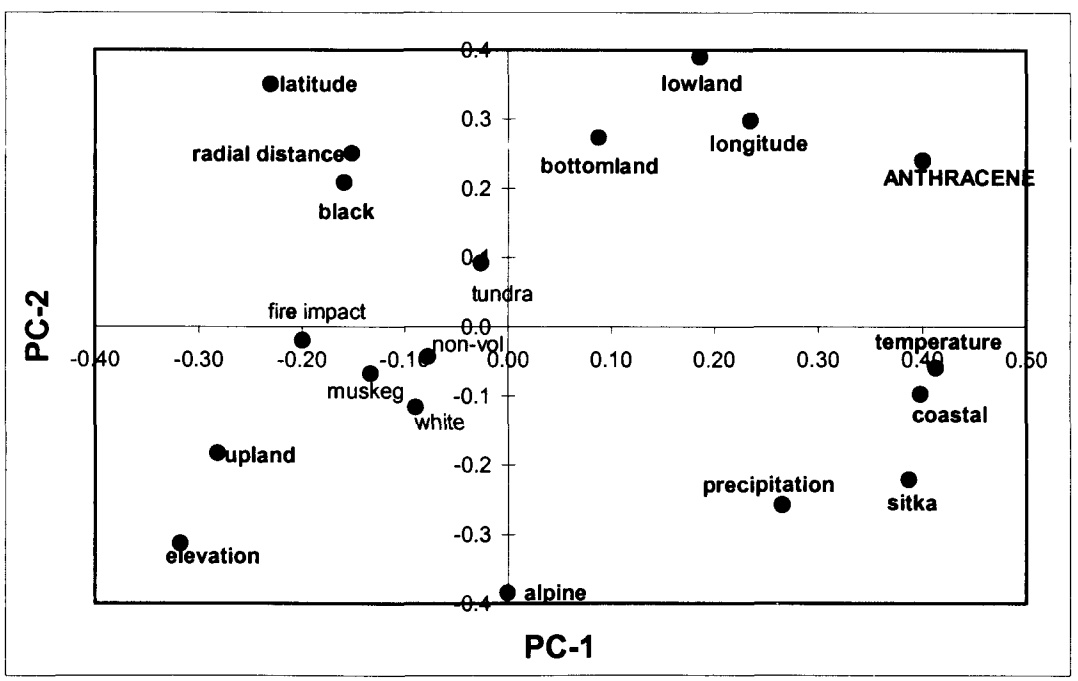


Figure 4.7. X loading weights and Y loadings for anthracene PLS-1 model. X explained 21%, 10%. Y explained 65%, 11%.

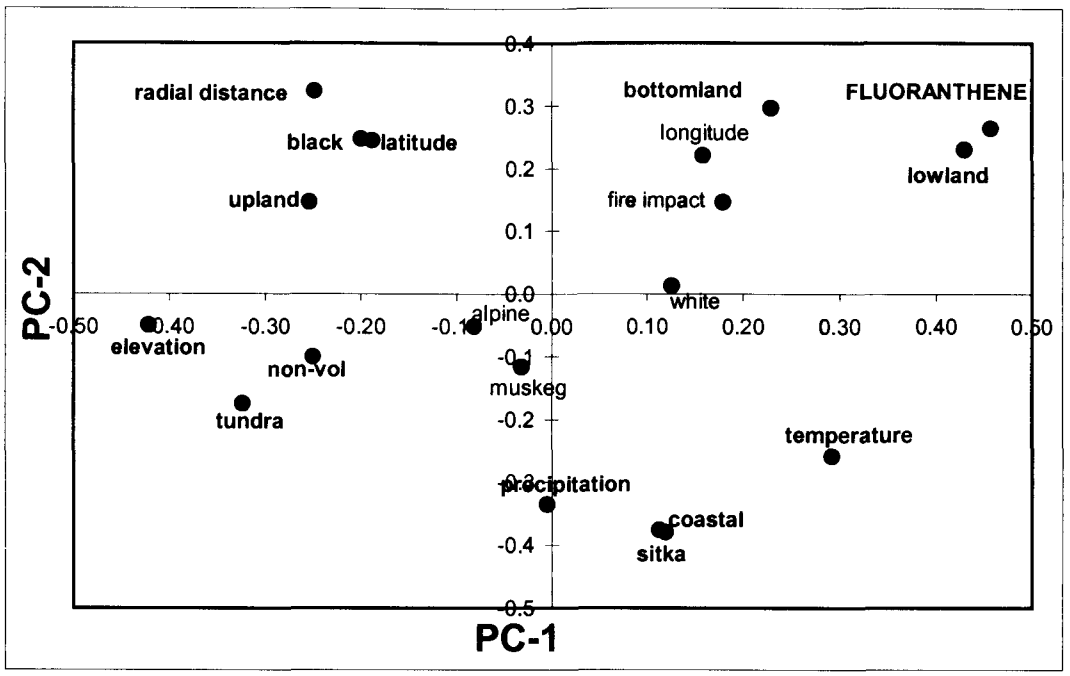


Figure 4.8. X loading weights and Y loadings for fluoranthene PLS-1 model. X explained 15%, 16%. Y explained 46%, 14%.

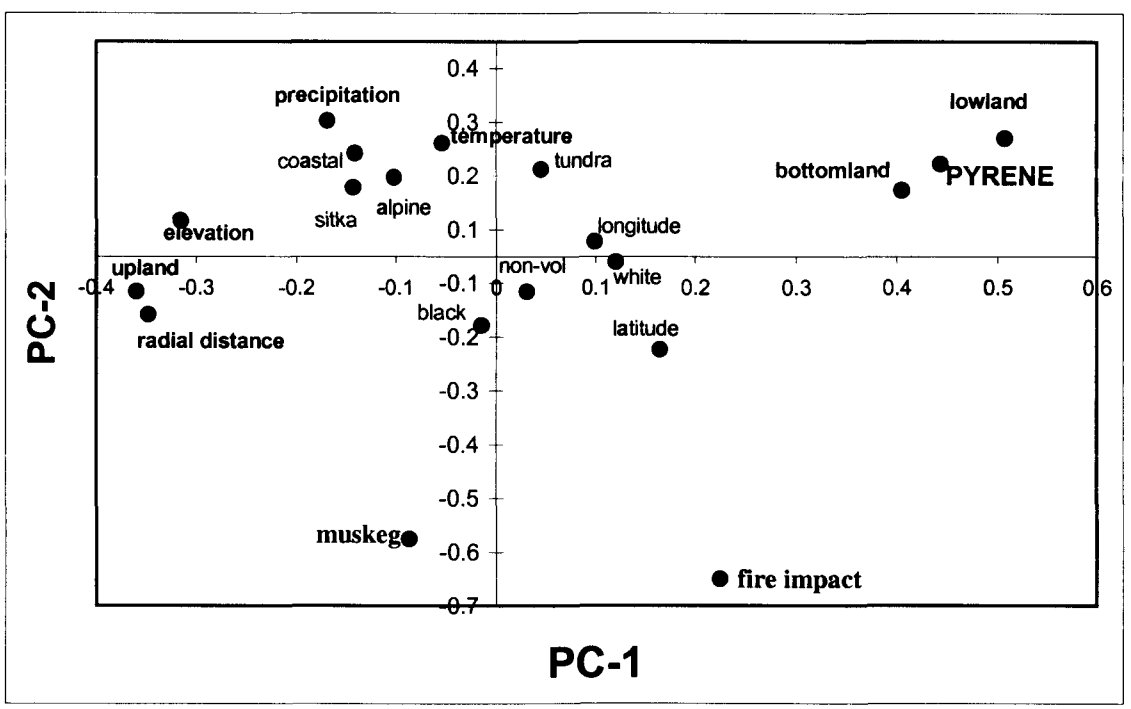


Figure 4.9. X loading weights and Y loadings for pyrene PLS-1 model. X explained 8%, 20%. Y explained 31%, 2%.

The variables in bold in Figures 4.6 through 4.9 are considered to be important variables. The delineation between important and unimportant variables is based on the spread of loading weights in the principle components. Variables with loading weights relatively far from the origin are considered important. Although some ecosystem variables are important in each model, physical interpretation of the data has been made using only non-ecosystem variables. Because Alaskan ecosystem delineation is largely determined by elevation we have chosen to interpret the results in the context of physical and geographical factors. Table 4.8 gives a summary of important variables for each model.

Table 4.8. Important Variables from PLS-1 Models.

Model Name	PC-1	PC-2
Phenanthrene	elevation, radial distance, temperature, non-volatile extractable content	latitude, precipitation
Anthracene	elevation, temperature, latitude, longitude, precipitation, radial distance	latitude, elevation, radial distance
Fluoranthene	radial distance, elevation, temperature, latitude	radial distance, precipitation, temperature, latitude
Pyrene	elevation, radial distance	n/a

At the bottom of Figures 4.6 - 4.9 is the explained variance. These values can be interpreted as the amount of the concentration data variance accounted for in the model by PC-1 and PC-2. For example, in the anthracene model, the figure caption reads, "X explained 21%, 10%. Y explained 65%, 11%". This means that for PC-1, 21% of the variance in the X-variables accounts for 65% of the variance in the anthracene concentrations. In PC-2 an additional 10% of the X-variance accounts for an additional 11% of the variation in the concentrations. This demonstrates a major advantage of multivariate analysis over univariate approaches. In the anthracene model only 31% of the X-variance is considered to account for over 75 % of the variance in the analyte concentration.

The next step in interpretation of the above models is to simplify the discussion by generalizing the results of each model. This is done by evaluating the correlations of the variables in Table 4.8 to the analyte concentrations. In Figures 4.6 – 4.9, variables in the same quadrant, such as temperature and precipitation, are positively correlated in PC-1 and PC-2. Variables within the same half of the plot, such as radial distance and elevation, are positively correlated in the PC running through both halves. Variables on opposite sides of the origin, such as elevation and bottomland are negatively correlated. Table 4.9 gives a generalized list of correlations between X-variables correlations and analyte concentrations.

Table 4.9. List of General Analyte Correlations to X-variables

Correlation Sign	PC #	Variables
Positive	1	temperature
	2	latitude
Negative	1	radial distance, elevation
	2	precipitation

According to Table 4.9 PAH concentrations tend to decrease with increasing radial distance, elevation and precipitation. Also, PAH concentrations tend to increase with increasing temperature and latitude. The most important trends in analyte concentration are described by elevation, temperature and radial distance from urban sites. These variables are strongly represented by PC-1, which accounts for the majority of the explained variances.

At this stage of model interpretation it is useful to review the score plots for the models discussed above. A score plot shows the distribution of samples in PC space. Figures 4.10 – 4.12 are score plots for phenanthrene, anthracene, and fluoranthene using PC-1 and -2. A score plot for the pyrene model is not included. The pyrene model is not a quality model. The amount of explained variance is considerably lower than the other models. While the other models have total explained Y-variance greater then 50% the

pyrene model only explains a total of 33%. It is likely that the pyrene model is not representative of true variable relationships.

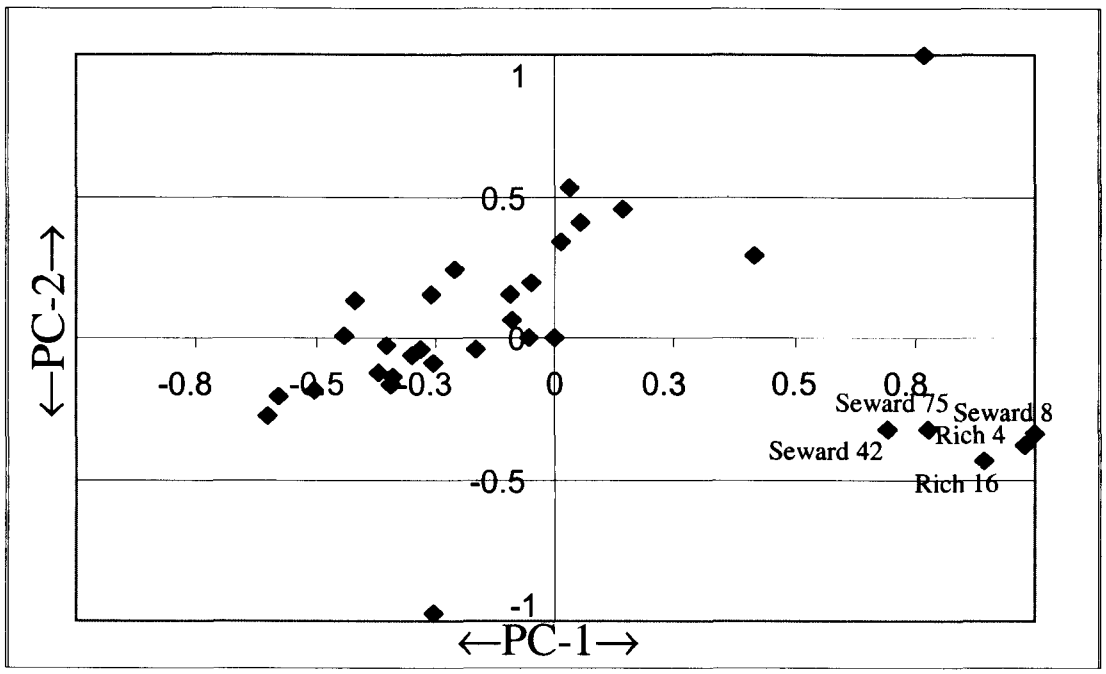


Figure 4.10. Score plot for phenanthrene PLS-1 model.

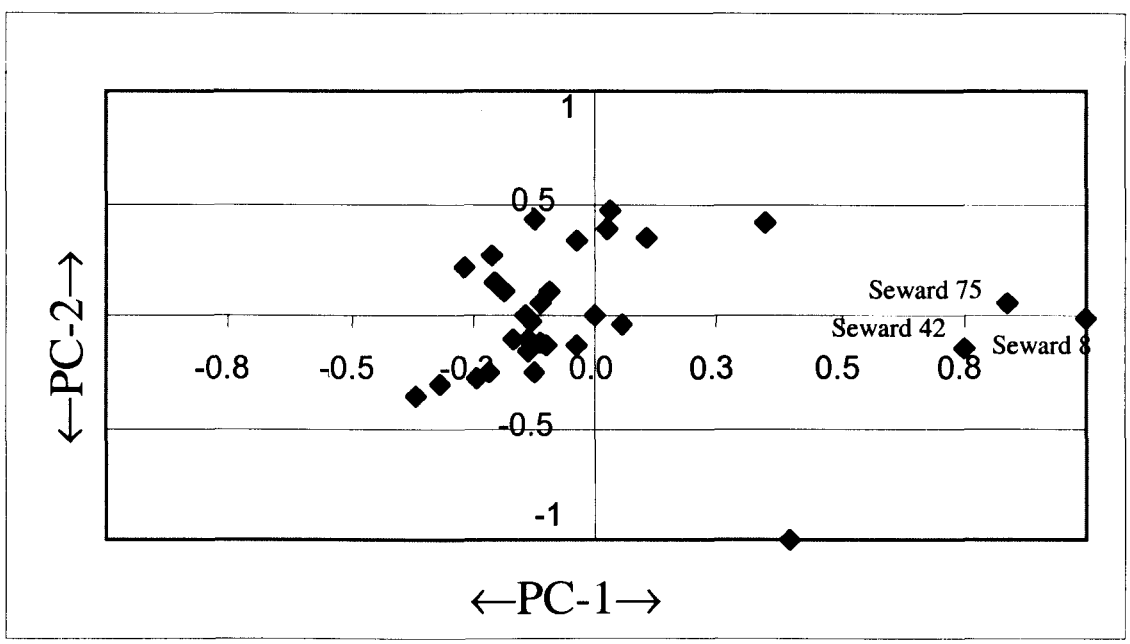


Figure 4.11. Score plot for anthracene PLS-1 model.

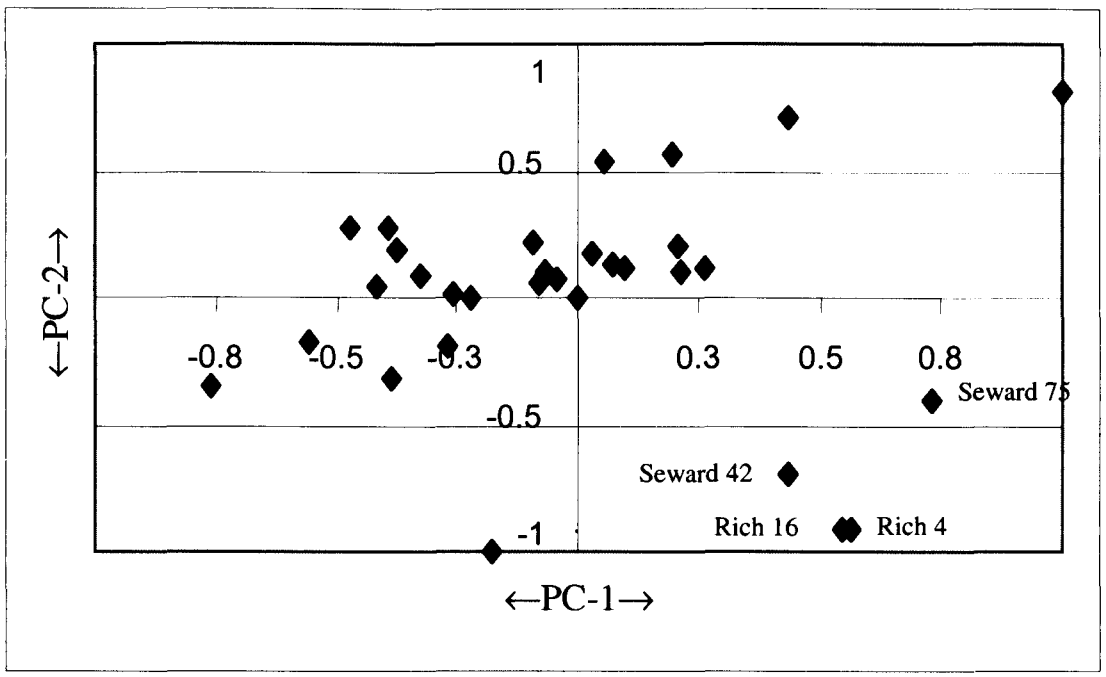


Figure 4.12. Score plot for fluoranthene PLS-1 model.

Figures 4.10-4.12 show evidence of clustering in the sample data. Clustering can be viewed as the separation of samples into different groups. Clustering can become a problem when a model describes the difference between two groups of samples without effectively describing the differences between samples within a group. In Figures 4.10-4.12 two distinct groups of samples can be seen, the coastal samples, represented by the Seward highway samples and Richardson 4 and 16, and the non-coastal samples. The coastal samples are characterized by relatively low latitudes, high precipitation and high temperatures. They are also among the samples with the highest analyte concentrations. This trend could be attributed to removal of PAHs as air moves in from the ocean. The ocean air is depleted of PAH as it moves into the mountains thereby causing high PAH levels in coastal spruce needles. It is therefore likely that the PLS-1 models are largely describing the differences between the few coastal samples and the rest of the samples.

Although differences between coastal and non-coastal samples are interesting results, far more satisfying models would describe trends within the coastal and non-coastal sample

sets. Separating the samples into geographical groups and modeling each group would achieve this goal. Of the five geographical areas in eastern Alaska (Interior Alaska, Matanuska-Susitna Valley, Kenai Peninsula, Copper River Valley, and Valdez Coast), only interior Alaska is well represented in our sample set. Twenty of the 35 samples are within interior Alaska (Table 4.10). Only three or four samples are within each of the other geographical areas

Table 4.10. List of Interior Alaska Samples

Sample Name	Direction from Fairbanks	Sample Name	Direction from Fairbanks
AK 1230	SE	Dalton 160	NW
AK 1260	SE	Dalton 193	NW
AK 1301	SE	Elliot 9	NW
AK 1345	SE	Elliot 47	NW
AK 1384	SE	Parks 230	SW
AK 1418	SE	Parks 270	SW
Dalton 13	NW	Rich 277	SE
Dalton 50	NW	Rich 309	SE
Dalton 87	NW	Rich 339	SE
Dalton 122	NW	Goldstream	NW

PLS-2 models were developed using interior Alaska samples. Since Fairbanks is the only urban site within interior Alaska, direction from Fairbanks is included as a categorical variable (Table 4.10). The PAH analytes were separated into two groups, 3-ring PAHs (phenanthrene and anthracene) and 4-ring PAHs (fluoranthene and pyrene). PLS-2 models were developed for each group of analytes using the parameters listed in Appendix E. The X-loadings and Y loading weights plot for these models are shown in Figures 4.13 –4.14.

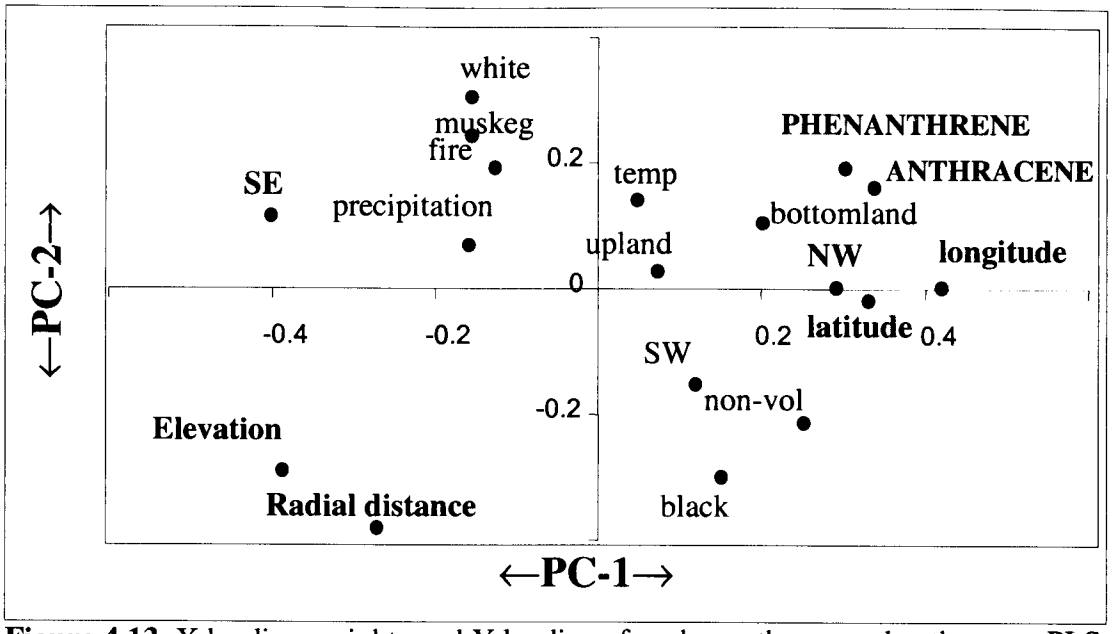


Figure 4.13. X loading weights and Y loadings for phenanthrene and anthracene PLS-2 model. X explained 24%, 18%. Y explained 44%, 4%.

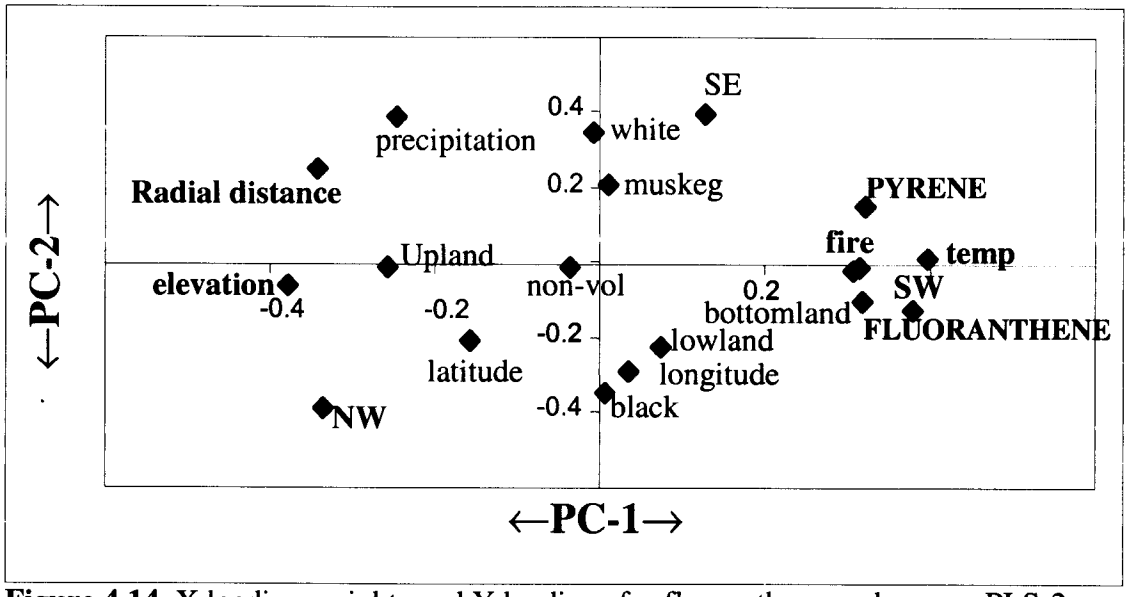


Figure 4.14. X loading weights and Y loadings for fluoranthene and pyrene PLS-2 model. X explained 18%, 24%. Y explained 39%, 2%.

In both models, PAH concentrations have a strong negative correlation with elevation and radial distance from Fairbanks (Figures 4.13 and 4.14). In other words, PAH concentrations increase as elevation and radial distance from Fairbanks decrease. Elevation and radial distance from Fairbanks are strongly correlated (Figure 4.15).

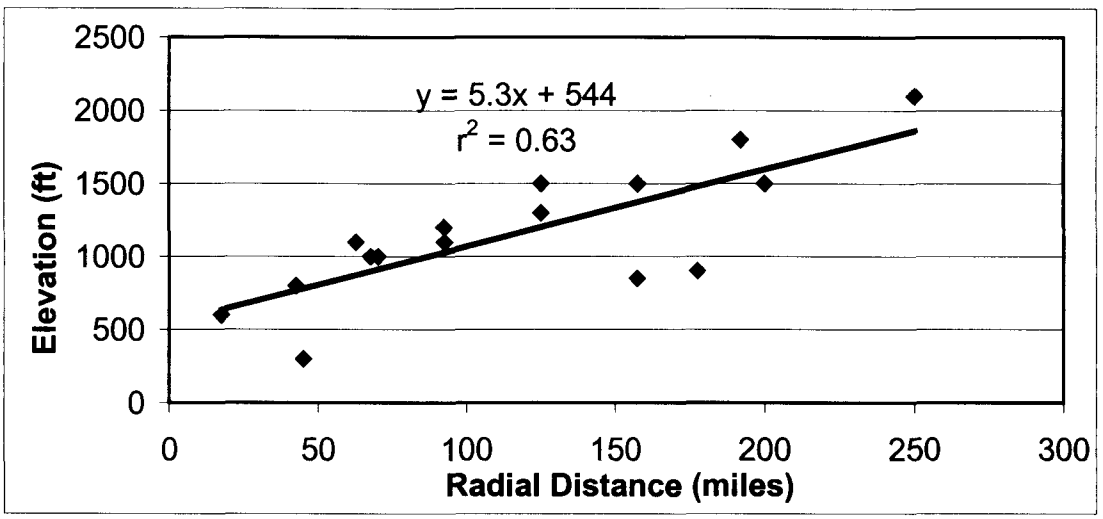


Figure 4.15. 2D scatter plot of elevation and radial distance from Fairbanks.

The importance of radial distance and elevation can be looked at in two ways. One, that only one of them is actually important and that the other, because of their strong correlation to each other, coincidentally appears important. The other and more likely scenario is that the both are important. Fairbanks is located in a valley between two mountain ranges, the Alaska Range to the south and the Brooks Range to north. Fairbanks experiences thermal inversion during the winter because it is located in this low-lying area surrounded by higher elevation. When thermal inversions occur, the air surrounding Fairbanks becomes trapped, allowing for urban pollution, including PAH, to accumulate. The PLS-1 models (Figures 4.6-4.9) and the PLS-2 models (Figures 4.13 and 4.14) support this phenomenon. Concentrations of PAHs tend to be highest at low elevations close to urban sites.

Another observation from Figures 4.13 and 4.14 is that there is a clear difference between 3 and 4-ring PAH distribution patterns. Samples north of Fairbanks tend to be higher in 3-ring PAH concentrations then south of Fairbanks. The opposite seems to occur with 4-ring PAHs. Samples south of Fairbanks tend to be higher in 4-ring PAH concentrations then in the north. This could be the result of differences in the sources of PAHs in interior Alaska. In these models two possible sources are accounted for, urban pollution and forest fires. Perhaps forest fires contribute more to 4-ring PAH concentrations then to 3-ring PAH concentrations. If this is the case then the most northern samples (Dalton highway) would be relatively lower in 4-ring PAHs then the southern interior Alaska samples. According to Figure 3.1 and Table 3.6 the Dalton highway samples have very low potential forest fire impact. Samples south of Fairbanks along the Parks, Richardson and Alaska highways have a much greater potential forest fire impact. According to Figure 4.13 forest fire impact is not important in regards to 3-ring PAHs. However, in Figure 4.14, forest fire impact has a strong positive correlation to 4-ring PAH concentrations.

In Figure 4.14, temperature has a positive correlation with 4-ring PAH concentrations. This indicates that 4-ring PAH concentrations tend to increase with increasing temperature. Although this may be true, it is most likely coincidence and a result of the correlation between forest fire impact and temperature (Figure 4.16).

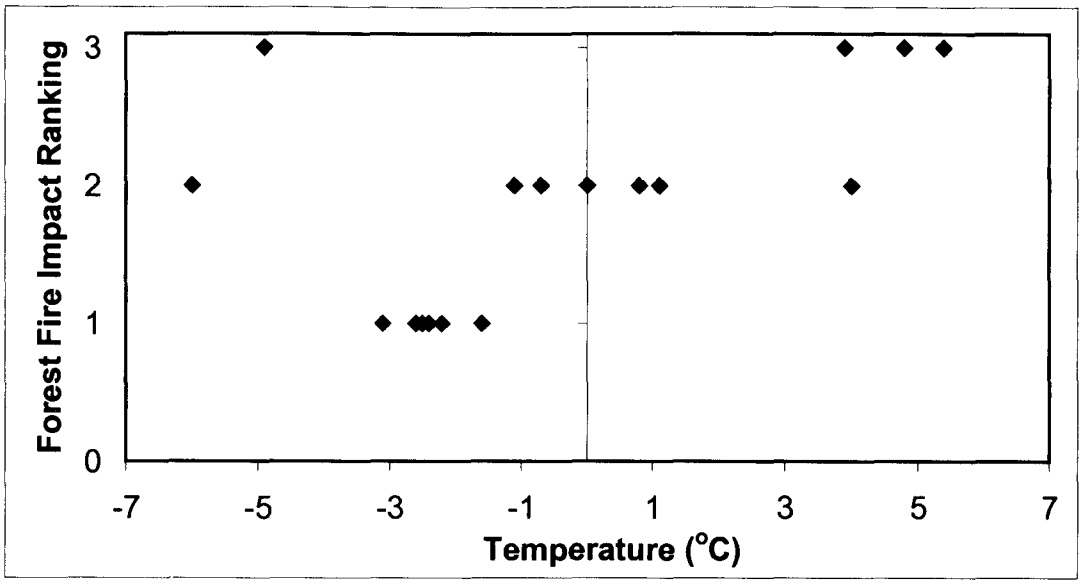


Figure 4.16. 2D scatter plot of forest fire impact ranking and temperature.

Figure 4.16 shows a clear trend of increasing temperature with increasing forest fire impact ranking. The warm temperature samples (> 0°C) have forest fire ranking of either 2 or 3, while the majority of cold temperature samples (< 0°C) have forest fire impact of 1.

There are also some interesting observations about non-volatile extractable content, which is often referred to as the lipid content of the needles. Hites and co-workers have stressed the importance of normalizing organic pollutant concentration in vegetation by lipid content, especially when comparing data from different species of plants (3). This indicates that there would be strong correlation between PAH concentration and lipid content. From the results of this study the correlation that Hites has alluded to is not obvious. According to Figures 4.6- 4.9 the correlation ranges from positive (phenanthrene) to no correlation (anthracene and pyrene) to negative (fluoranthene). This would indicate that the lipid content measurements is not very important in the determination of PAH concentration in Alaskan spruce needles. Although Hites'

approach might hold true when comparing moss to birch bark, it does not seem to be the best approach when studying PAH content of Alaskan spruce species needles.

In conclusion, PAH concentrations are strongly correlated to elevation and radial distance from urban site. The general trend shows that PAH concentrations increase as elevation and radial distance from urban site decrease. When considering all samples from eastern Alaska, coastal samples tended to have higher PAH concentrations then non-coastal samples indicating ocean air as a possible PAH source. The interior Alaska models also indicated forest fire as a possible source of 4-ring PAHs. A summary of the models indicated that three possible sources of PAHs exist in eastern Alaska, urban sites (Fairbanks, Anchorage and Valdez), ocean air, and forest fires. Distribution of PAHs away from these sources is strongly correlated with elevation.

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Appendix A. Map of Eastern Alaska.

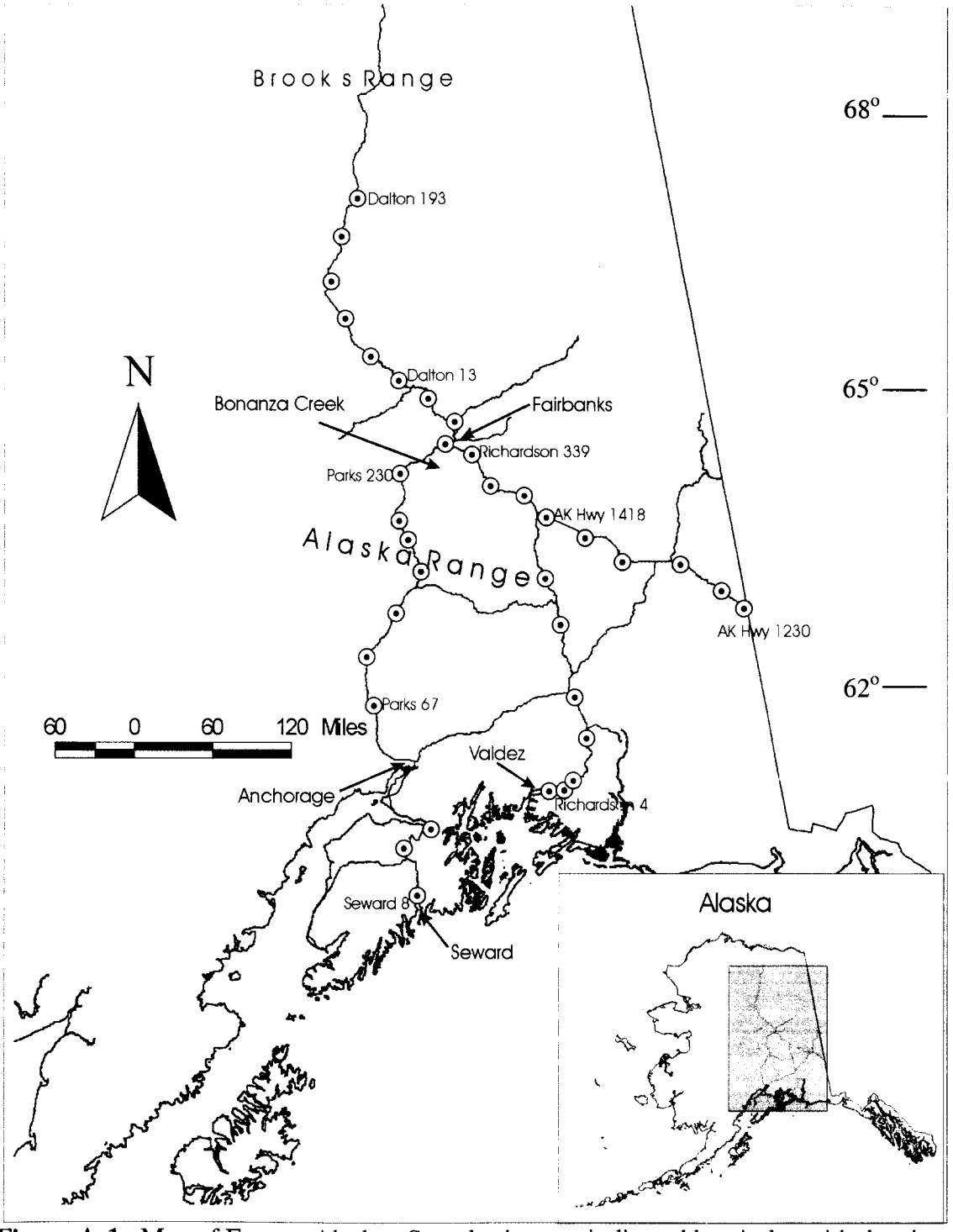

Figure A-1. Map of Eastern Alaska. Sample sites are indicated by circles with dots in the middle.

Appendix B. Forest Fire Information.

Table B-1. Forest Fire Information.

Forest Fires 1995-1996*							
date	**acreage**	**longitude**	**latitude**	**date**	**acreage**	**longitude**	**latitude**
5/29/96	66560	146.39	63.93	6/16/96	204	147.28	65.36
6/17/96	59063	151.92	64.87	6/18/96	150	148.27	64.48
6/19/96	41410	148.00	66.77	7/20/96	150	141.65	62.63
7/11/96	39660	146.64	65.95	5/7/95	140	147.60	64.80
6/16/96	39116	144.02	65.53	7/28/95	100	151.72	66.72
6/2/96	37336	149.78	61.60	4/22/95	90	144.97	63.97
6/18/96	34665	143.98	65.75	7/6/96	88	150.13	64.52
6/19/96	33490	146.48	66.54	6/18/96	80	144.75	62.85
6/17/96	25433	151.35	64.27	7/2/96	70	151.38	67.12
5/10/96	14200	146.49	63.95	6/12/95	70	145.97	62.63
6/3/96	12760	144.90	65.57	5/12/95	62	142.80	63.20
6/15/96	10331	147.58	65.60	7/4/96	60	149.47	64.18
7/17/95	8500	147.80	65.33	6/15/96	60	147.88	64.35
7/14/95	7500	149.57	64.77	6/6/96	60	146.43	64.72
6/7/96	6710	142.42	63.25	7/24/96	50	146.55	63.93
6/17/96	5630	148.92	65.83	5/25/96	40	146.17	64.63
5/3/95	2960	145.27	63.97	4/25/95	35	145.58	64.08
6/7/96	2000	146.25	64.03	6/20/96	35	145.03	62.75
5/12/95	1880	142.03	63.45	5/13/95	30	146.07	64.75
6/8/96	1800	145.16	63.90	5/4/96	20	150.73	62.33
6/14/95	1200	145.29	63.97	7/4/96	20	144.77	62.33
6/12/95	1060	144.72	62.50	6/16/96	15	147.07	64.15
7/4/96	350	150.12	63.85	6/13/95	15	144.98	62.77
6/21/95	330	148.98	65.57	5/10/96	10	149.95	62.27
4/22/95	326	144.98	64.02	7/8/96	10	147.40	65.18
5/12/95	320	147.82	65.40	9/20/95	10	146.82	64.70
6/24/96	261	145.53	64.02	5/14/96	10	144.27	61.53

*Data obtained from Mary Lynch at Bureau of Land Management.

Appendix C. Sample Information and Data.

Table C-1. Sample Extraction Information.

Sample ID	sequence date	Sequence Run order	needle mass (g)	extraction date	non-volatile (mg)	Water (%)	final ext. vol. (µL)	Int. std. Conc. (µg/L)
Ak 1260.2	11-Feb	10	9.978	11/03/97	490.2	50.2	410	307
Rich 109.1	11-Feb	3	10.007	11/03/97	194.4	52.9	350	307
Dal 13.1a	11-Feb	5	9.987	11/03/97	169.2	50.2	295	307
Dal 13.1b	11-Feb	6	9.985	11/03/97	184.2	50.2	225	307
Sew 8.2a	4-Mar	7	10.009	11/03/97	167.2	54.4	410	307
Sew 8.2b	25-Feb	1	9.943	11/03/97	193.1	54.4	320	307
Dal 160.2a	25-Feb	8	9.977	11/18/97	181.3	46.8	180	307
Dal 160.2b	23-Feb	4	9.986	11/18/97	202.3	46.8	275	307
Nov18 blank	11-Feb	7	n/a*	11/18/97	n/a	n/a	280	307
Ak 1301.1a	4-Mar	9	9.998	11/18/97	105.8	52.3	260	307
Ak 1301.1b	23-Feb	3	9.964	11/19/97	82.7	52.3	328	307
Ak 1301.2a	25-Feb	3	9.993	11/19/97	106.8	49.8	320	307
Ak 1301.2b	25-Feb	10	9.978	11/19/97	136.4	49.8	280	307
Nov19 blank	25-Feb	5	n/a	11/19/97	n/a	n/a	155	307
Dal 160.1a	11-Feb	9	9.926	11/19/97	257.6	50.4	330	307
Dal 160.1b	11-Feb	2	9.95	11/19/97	236.6	50.4	300	307
Rich 207.2a	11-Feb	4	9.293	11/26/97	115.5	51.8	265	307
Rich 207.2b	11-Feb	1	9.963	11/26/97	140.8	51.8	310	307
Ell 9.1a	11-Feb	8	9.986	11/26/97	78.4	52.1	415	307
Ell 9.1b	4-Mar	3	9.961	11/26/97	106.2	52.1	390	307
Rich 109.2	23-Feb	9	10.005	11/26/97	113.9	50.2	280	307
Dal 13.2a	25-Feb	7	9.989	12/10/97	140.2	51.4	355	307
Dal 13.2b	4-Mar	5	10.008	12/10/97	128.8	51.4	280	307
Rich 339.2a	25-Feb	2	9.962	12/10/97	165.6	45.5	250	307
Rich 339.2b	23-Feb	10	9.981	12/10/97	156.7	45.5	283	307
Sew 75.2a	23-Feb	1	9.76	12/10/97	97.6	52.4	428	307
Rich 309.1sp	23-Feb	6	9.955	12/12/97	126.9	52.2	440	307
Rich 309.1	4-Mar	4	9.957	12/12/97	122.6	52.2	348	307
Sew 75.2b	23-Feb	2	9.915	12/12/97	62.9	52.4	230	307
Rich 31.1a	25-Feb	4	9.965	12/12/97	122.6	50.9	259	307
Rich 31.1b	25-Feb	9	9.978	12/12/97	974.2	50.9	385	307
Dec12 blank	27-Feb	9	n/a	12/12/97	n/a	n/a	335	307
Dec10 blank	4-Mar	8	n/a	12/10/97	n/a	n/a	240	307
Jan29 blank	23-Feb	7	n/a	1/29/98	n/a	n/a	329	307
Rich 339.1b	23-Feb	8	9.949	1/29/98	278.5	46.8	370	307
Rich 31.2a	23-Feb	5	9.939	1/29/98	98.7	49.9	330	307
Rich 31.2b	11-Mar	2	9.988	1/29/98	103.9	49.9	280	307
Ell 47.1	8-Mar	6	9.966	2/3/98	344.7	47.3	210	246
Ell 47.1sp	8-Mar	5	9.887	2/3/98	337.3	47.3	342	246

Table C-1. (con't)

Sample ID	sequence date	Sequence Run order	needle mass (g)	extraction date	non-volatile (mg)	Water (%)	final ext. vol. (µL)	Int. std. Conc. (µg/L)
Ak 1418.1	27-Feb	8	9.883	2/3/98	184.1	48.9	230	246
Ak 1418.1sp	8-Mar	2	9.917	2/3/98	179.7	48.9	344	246
Park 67.1	8-Mar	1	9.922	2/3/98	97.0	53.7	344	246
Park 147.1a	4-Mar	6	9.976	2/11/98	73.4	54.0	345	246
Park 147.1b	8-Mar	8	9.947	2/11/98	77.9	54.0	368	246
Park 67.2	27-Feb	3	9.902	2/11/98	97.2	54.6	245	246
Ell 9.2a	8-Mar	7	10.001	2/11/98	169.8	50.1	340	246
Ell 9.2b	8-Mar	4	9.978	2/11/98	189.2	50.1	262	246
Feb11 blank	2-Mar	10	n/a	2/11/98	n/a	n/a	352	246
Feb12 blank	2-Mar	3	n/a	2/12/98	n/a	n/a	312	246
Park 147.2a	27-Feb	10	9.958	2/12/98	121.7	51.5	291	246
Park 147.2b	11-Mar	4	9.923	2/12/98	122.8	51.5	304	246
Ak 1418.2	2-Mar	5	9.999	2/12/98	145.4	50.6	255	246
Ak 1418.2sp	27-Feb	7	9.944	2/12/98	150.3	50.6	390	246
Dal 193.1	2-Mar	9	9.958	2/12/98	146.4	52.8	310	246
Feb13 blank	25-Feb	6	n/a	2/12/98	n/a	n/a	214	246
Dal 193.1sp	27-Feb	6	9.973	2/12/98	218.4	52.8	222	246
Ak 1260.1	27-Feb	5	9.933	2/12/98	568.7	46.7	370	246
Dal 193.2	11-Mar	3	9.927	2/12/98	130.7	48.1	238	246
Dal 193.2sp	27-Feb	4	9.925	2/12/98	127.9	48.1	272	246
Rich 339.1a	27-Feb	2	9.935	2/12/98	324.5	46.8	253	246
Feb18 blank	2-Mar	4	n/a	2/18/98	n/a	n/a	425	246
Dal 87.1	8-Mar	10	9.867	2/18/98	166.4	54.8	338	246
Rich 16.1	4-Mar	2	9.918	2/18/98	173.5	52.3	379	246
Rich 16.1sp	4-Mar	1	9.993	2/18/98	184.0	52.3	385	246
Sew 42.2	2-Mar	1	9.955	2/18/98	188.1	51.5	385	246
Sew 42.2sp	2-Mar	7	10.004	2/18/98	199.3	51.5	240	246
Feb20 blank	27-Feb	1	n/a	2/20/98	n/a	n/a	400	246
Rich 277.1	2-Mar	8	9.878	2/20/98	107.2	53.3	400	246
Sew 42.1	2-Mar	6	9.94	2/20/98	158.7	53.2	380	246
Sew 42.1sp	8-Mar	9	9.879	2/20/98	170.6	53.2	320	246
Rich 309.2	2-Mar	2	9.904	2/20/98	159.3	48.2	362	246
Rich 309.2sp	11-Mar	5	9.945	2/20/98	152.6	48.2	255	246
Rich 16.2	11-Mar	1	10.004	2/26/98	173.5	51.2	210	246
Rich 16.2sp	11-Mar	9	10.005	2/26/98	163.3	51.2	368	246
Feb26 blank	11-Mar	10	n/a	2/26/98	n/a	n/a	350	246
Rich 277.2	3-Apr	2	9.946	2/26/98	145.6	51.5	362	246
Park 230.2	1-Apr	7	9.805	2/26/98	138.0	51.6	272	246
Park 230.2sp	11-Mar	7	9.916	2/26/98	131.9	51.6	388	246
Park 214.1	11-Mar	6	9.896	2/26/98	147.4	52.4	295	246
Park 214.2	3-Apr	8	9.932	2/26/98	146.4	48.4	286	246
Ak 1384.1	3-Apr	3	9.7	2/26/98	106.7	49.5	324	246

Table C-1. (con't)

Sample ID	sequence date	Sequence Run order	needle mass (g)	extraction date	non-volatile (mg)	Water (%)	final ext. vol. (µL)	Int. std. Conc. (µg/L)
Ak 1384.2	1-Apr	6	9.9	2/26/98	78.0	49.2	270	246
Park 270.1a	1-Apr	8	9.867	2/26/98	345.8	50.4	255	246
Park 270.1b	11-Mar	8	9.964	2/26/98	358.8	50.4	392	246
Mar3 blank	1-Apr	4	n/a	3/3/98	n/a	n/a	250	246
Ak 1230.2	3-Apr	7	9.933	3/3/98	141.3	51.5	288	246
Ak 1230.2sp	3-Apr	5	9.916	3/3/98	155.9	51.2	285	246
Ak 1230.1	1-Apr	3	9.974	3/3/98	183.3	50.5	342	246
Ak 1230.1sp	1-Apr	1	9.821	3/3/98	179.3	50.9	384	246
Dal 87.2	1-Apr	10	9.949	3/3/98	166.4	51.5	162	246
Rich 72.2	3-Apr	9	9.94	3/3/98	160.4	49.1	244	246
Dal 122.2	1-Apr	5	9.937	3/3/98	306.7	50.7	162	246
Dal 50.1	1-Apr	2	9.904	3/3/98	384.0	51.3	360	246
Dal 50.1sp	3-Apr	4	9.928	3/3/98	392.4	51.3	292	246
Sew 8.1a	3-Apr	1	9.935	3/3/98	251.3	50.8	254	246
Sew 8.1b	1-Apr	9	9.912	3/10/98	234.0	50.8	310	246
Mar10 blank	8-Apr	1	n/a	3/10/98	n/a	n/a	205	246
Rich 72.1	8-Apr	5	9.99	3/10/98	220.1	48.6	375	246
Dal 50.2	9-Apr	6	9.985	3/10/98	329.3	50.9	265	246
Dal 50.2sp	8-Apr	9	9.945	3/10/98	330.0	50.9	200	246
Park 107.2	8-Apr	4	9.904	3/10/98	256.0	49.7	340	246
Park 107.2sp	8-Apr	7	9.992	3/10/98	263.7	49.7	330	246
Rich 4.1	3-Apr	10	10.014	3/11/98	248.4	50.6	375	246
Rich 4.2	9-Apr	1	10.015	3/11/98	166.8	52.8	250	246
Dal 122.1	9-Apr	4	9.971	3/11/98	363.5	49.6	375	246
Rich 166.2	9-Apr	3	10.003	3/11/98	302.4	49.6	260	246
Rich 166.2	8-Apr	10	9.998	3/11/98	284.2	49.6	275	246
Park 187.1	8-Apr	8	9.965	3/11/98	98.2	51.2	350	246
Mar11 blank	8-Apr	3	n/a	3/11/98	n/a	n/a	365	246
Park 187.2	3-Apr	6	9.995	3/11/98	114.0	46.4	335	246
Ak 1345.1	9-Apr	5	9.99	3/11/98	97.9	51.8	255	246
Ak 1345.2	8-Apr	6	9.783	3/11/98	79.7	50.3	375	246
Park 107.1	8-Apr	2	9.998	3/11/98	84.6	49.4	310	246
Park 107.1sp	9-Apr	2	9.689	4/7/98	70.0	49.4	450	246
Rich 166.1	13-Apr	8	9.841	4/7/98	172.3	50.6	645	246
Rich 166.1sp	13-Apr	7	9.919	4/7/98	166.1	50.6	449	246
Park 230.1	9-Apr	9	9.896	4/7/98	176.0	50.5	294	246
Park 230.1sp	13-Apr	3	9.851	4/7/98	170.0	50.5	450	246
Park270.2a	9-Apr	8	9.984	4/7/98	419.0	49.5	330	246
Park 270.2b	9-Apr	10	9.963	4/7/98	429.2	49.5	256	246
Ell 47.2	13-Apr	2	9.89	4/7/98	320.9	49.3	280	246
Ell 47.2sp	13-Apr	6	9.97	4/7/98	319.8	49.3	290	246
Gold 1	13-Apr	4	9.995	4/7/98	175.5	50.6	321	246

Table C-1. (con't)

Sample ID	sequence date	Sequence Run order	needle mass (g)	extraction date	non-volatile (mg)	Water (%)	final ext. vol. (μL)	Int. std. Conc. (μg/L)
Gold 2	13-Apr	5	9.926	4/7/98	161.3	48.4	470	246
Apr7 blank	9-Apr	6	n/a	4/7/98	n/a	n/a	288	246
Nov26 blank	4-Mar	10	n/a	11/26/97	n/a	n/a	260	307

* method blanks did not contain needles therefore needle mass, non-volatile mass and % water have no data.

Table C-2. Peak Areas from Sample Extraction Chromatograms.

Sample ID	Fluorene	Phen-anthrene	Anthra-cene	Phenan-threne-d10	Fluor-anthene	Pyrene	Benzo(a) anthracene	Chrysene	Chrys-ene- d12
Ak 1260.2	n/a*	39794	6095	16087	10499	2923	n/a	n/a	n/a
Rich 109.1	n/a	45972	1133	28248	12135	11138	881	4341	9649
Dal 13.1a	n/a	36064	1243	26062	7167	4033	1236	2201	41296
Dal 13.1b	n/a	52591	1127	30665	6680	7484	1299	3108	29702
Sew 8.2a	4041	31065	1972	18778	n/a	11209	611	3507	8992
Sew 8.2b	20860	115839	7917	40073	29945	35157	2239	10788	20291
Dal 160.2a	17300	68692	3163	39698	10259	8396	537	21354	17986
Dal 160.2b	13311	31798	905	27609	6345	12001	1049	13900	10732
Nov18 blank	7428	17196	807	23982	3333	8781	502	733	10283
Ak 1301.1a	18169	93559	3437	23348	13089	14545	800	4633	11994
Ak 1301.1b	6368	19025	585	28378	4465	4386	312	3713	14194
Ak 1301.2a	12252	48325	3736	40488	12391	17197	1605	5404	17185
Ak 1301.2b	15862	58263	1403	31679	10920	8693	382	2544	14302
Nov19 blank	16271	70453	3758	70965	13234	13328	389	1937	42279
Dal 160.1a	8985	24808	831	17452	5775	4580	n/a	15480	7353
Dal 160.1b	18652	45339	1824	33027	10562	14849	410	19602	12122
Rich 207.2a	20788	80520	3126	47765	25145	31388	933	5631	17142
Rich 207.2b	7754	32280	811	32302	7702	6577	180	792	19148
Ell 9.1a	5146	19501	537	18478	4823	4295	163	1202	8069
Ell 9.1b	5282	22028	414	18275	4400	5111	322	1816	9499
Rich 109.2	3898	38572	2416	23114	6760	4936	368	1752	11441
Dal 13.2a	5064	22639	395	30594	6115	4148	171	1822	14645
Dal 13.2b	6674	27609	1408	25370	1532	5174	218	1923	13534
Rich 339.2a	12905	65789	897	39663	39815	29787	1600	7164	18755
Rich 339.2b	7407	46444	1582	26873	23000	15598	1247	4755	11862
Sew 75.2a	4733	20011	1014	24557	7112	5021	476	2605	13503
Rich 309.1sp	37161	63842	39134	19257	42518	43584	21719	26241	10275
Rich 309.1	5893	26211	840	21640	5174	7200	840	3092	11145
Sew 75.2b	14122	73473	5746	57545	14898	15219	993	6589	28377
Rich 31.1a	8080	27942	1267	36989	6202	5368	364	1898	18398
Rich 31.1b	5441	16786	834	24600	3810	3190	208	1127	12676
Dec12 blank	4562	21727	941	27595	3189	2876	n/a	515	15274

Table C-2. (con't)

Sample ID	Fluorene	Phen-anthrene	Anthra-cene	Phenan-threne-d10	Fluor-anthene	Pyrene	Benzo(a) anthracene	Chrysene	Chrys-ene- d12
Dec10 blank	4366	19370	798	28593	3045	2555	413	527	17661
Jan29 blank	4990	13794	548	29284	2399	5970	n/a	639	18566
Rich 339.1b	12376	75674	1057	21469	36311	23480	2853	21482	12239
Rich 31.2a	8755	29546	707	27211	5926	9224	337	1530	12415
Rich 31.2b	4052	55640	3039	63882	20562	12973	4052	1138	35459
Feb3 blank	5444	27140	1503	27617	27617	4778	4579	n/a	16036
Ell 47.1	n/a	66129	640	49342	49342	10833	9072	942	19778
Ell 47.1sp	2732	113504	47621	67044	67044	57981	53616	25807	30906
Ak 1418.1	13345	66177	1802	32100	11787	9503	296	2029	14533
Ak 1418.1sp	56486	117947	57558	77066	77066	69964	69253	35141	40052
Park 67.1	14058	70809	3824	43315	43315	25945	22835	1170	24749
Park 147.1a	10564	37588	1236	19723	7400	11064	1170	2160	9937
Park 147.1b	11856	40825	2299	29626	29626	9512	7737	369	13559
Park 67.2	13293	57695	2942	38201	18874	17470	775	6528	19212
Ell 9.2a	10024	43404	2218	33232	33232	14340	11414	550	15839
Ell 9.2b	13867	55424	2851	43070	43070	16691	16247	558	22275
Feb11 blank	5372	22212	825	21068	3585	3488	163	606	11175
Feb12 blank	4816	23118	735	27089	3646	4340	n/a	677	15803
Park 147.2a	11859	33377	562	29702	6152	9035	480	7092	12968
Park 147.2b	582	38295	836	36795	8209	7964	582	8475	18698
Ak 1418.2	14776	38942	920	33453	8668	8016	373	10931	16623
Ak 1418.2sp	33134	47595	30632	18378	30467	30985	15704	18916	9548
Dal 193.1	5487	34223	1544	32921	6360	5154	273	4236	15702
Feb13 blank	7283	17412	391	41025	3497	11106	705	832	24819
Dal 193.1sp	50334	98629	60829	77143	60913	64468	29712	30233	33532
Ak 1260.1	6424	46037	3703	21169	9363	14279	n/a	34183	n/a
Dal 193.2	449	35684	1012	53158	6121	7322	449	1585	26304
Dal 193.2sp	46539	77969	49938	61801	47080	49876	26801	25696	29743
Rich 339.1a	1484	221960	4125	38212	102606	77174	4764	52998	17517
Feb18 blank	1939	6317	93	3346	1221	1039	n/a	107	2010
Dal 87.1	12617	44540	731	32464	32464	7868	15679	121	14259
Rich 16.1	9798	23296	n/a	33095	5983	11596	n/a	3550	24389
Rich 16.1sp	26800	46547	30058	36541	30256	29973	18935	20632	25744
Sew 42.2	6550	30697	1097	25719	6460	4652	312	2764	12444
Sew 42.2sp	36998	64437	40906	55517	42425	38431	19911	19524	24389
Feb20 blank	4054	15824	496	26253	2772	3088	n/a	494	17505
Rich 277.1	7856	25627	355	21399	6752	12806	282	1657	10018
Sew 42.1	7087	26339	1308	21521	5788	4571	356	3514	10310
Sew 42.1sp	38488	70205	42909	60654	60654	46486	45176	22900	26799
Rich 309.2	7087	35064	400	26753	9181	16196	786	4546	13083
Rich 309.2sp	38987	126587	72623	89990	84386	85936	38987	40210	42169
Rich 16.2	1086	110495	2930	76679	12701	16798	1086	26334	46942

Table C-2. (con't)

Sample ID	Fluorene	Phen-anthrene	Anthra-cene	Phenan-threne-d10	Fluor-anthene	Pyrene	Benzo(a) anthracene	Chrysene	Chrys-ene- d12
Rich 16.2sp	22628	66432	43713	27619	44877	47748	22628	22043	11584
Feb26 blank	n/a	6760	173	31021	1260	1456	n/a	251	17469
Rich 277.2	16144	29783	814	48854	12494	12064	775	24988	26875
Park 230.2	35644	33099	n/a	35644	8481	8330	447	447	13841
Park 230.2sp	24212	69046	44107	28811	51757	53603	24212	18527	12082
Park 214.1	405	26775	134	37228	7545	1634	405	3065	16702
Park 214.2	10534	47416	1093	47983	11528	10359	484	8482	22183
Ak 1384.1	6656	32524	970	50382	6798	6569	578	7947	24752
Ak 1384.2	51493	38456	n/a	51493	7794	8632	697	697	20751
Park 270.1a	32513	66074	1205	32513	10494	8507	n/a	n/a	11479
Park 270.1b	457	82119	841	38002	10667	14004	457	15320	12589
Mar3 blank	43248	14141	356	43248	2662	3989	109	109	20148
Ak 1230.2	6598	38330	542	47741	6472	6625	385	4971	19143
Ak 1230.2sp	51293	100291	63838	44063	64734	68288	33280	37034	20350
Ak 1230.1	34725	22915	n/a	34725	2388	4666	246	246	14557
Ak 1230.1sp	40677	91351	58487	40677	68796	64000	34608	34608	18178
Dal 87.2	49728	28726	n/a	49728	4783	7032	n/a	n/a	17966
Rich 72.2	8112	39986	603	59427	5577	6133	218	15749	26308
Dal 122.2	44988	19237	815	44988	3709	3046	n/a	n/a	17930
Dal 50.1	34697	24716	1135	34697	2023	2733	n/a	n/a	15736
Dal 50.1sp	n/a	97959	56859	42569	54343	61234	19771	61121	12513
Sew 8.1a	20433	44593	894	49219	17086	12894	1089	8330	28470
Sew 8.1b	35903	28713	555	35903	11459	6852	704	704	13800
Mar10 blank	14616	61150	1027	80269	11358	8332	479	822	38420
Rich 72.1	7985	46883	209	37983	6894	5513	n/a	1596	13538
Dal 50.2	15716	61487	151	45679	10858	6507	n/a	6127	13837
Dal 50.2sp	53970	123245	62075	44167	56635	62374	21029	21801	11471
Park 107.2	9235	54602	210	37132	10022	6557	192	15417	11804
Park 107.2sp	52650	122610	48173	34733	62634	54022	20599	28683	9279
Rich 4.1	n/a	36710	611	37361	16380	19712	n/a	n/a	n/a
Rich 4.2	4886	43532	818	53838	15706	26322	n/a	n/a	n/a
Dal 122.1	7052	29557	469	34466	1509	8039	n/a	16351	10400
Rich 166.2	10646	42666	n/a	51295	1530	5197	n/a	14762	16296
Rich 166.2	49276	105577	61033	43554	56791	68843	15309	25297	10713
Park 187.1	6783	42860	n/a	47595	6729	4797	n/a	5154	13548
Mar11 blank	7456	31399	298	42042	5604	3943	135	355	19293
Park 187.2	14947	52142	678	49929	15256	21067	383	5359	21381
Ak 1345.1	13794	48119	671	48229	12202	16731	n/a	3546	12188
Ak 1345.2	2706	33323	450	46604	8595	16001	189	2640	13635
Park 107.1	6736	36933	680	59827	9766	8351	388	3939	18812
Park 107.1sp	42751	76844	43622	32900	47873	57263	18705	16360	10623
Rich 166.1	4469	19175	n/a	20646	3759	7061	n/a	410	6609

Table C-2. (con't)

Sample ID	Fluorene	Phen-anthrene	Anthra-cene	Phenan-threne-d10	Fluor-anthene	Pyrene	Benzo(a) anthracene	Chrysene	Chrys-ene- d12
Rich 166.1sp	45226	65971	41602	31559	46721	53869	19447	15598	10436
Park 230.1	12063	50886	622	45003	13044	9770	133	1504	12548
Park 230.1sp	40905	73451	38179	27256	47488	52869	19278	15258	9712
Park270.2a	n/a	55318	1288	39024	10289	18218	n/a	19726	9715
Park 270.2b	n/a	65254	1298	34594	10661	14891	n/a	19580	8428
Ell 47.2	16805	55958	691	23581	8403	12971	353	15904	7270
Ell 47.2sp	47941	76000	45630	33159	47595	54045	16539	31189	12584
Gold 1	11735	75459	998	24780	38642	26900	671	6956	10307
Gold 2	10183	58381	671	31085	43278	37263	684	7056	11044
Apr7 blank	5874	22994	278	50101	3535	2634	n/a	311	21022
Nov26 blank	3914	20052	587	23209	3346	3040	n/a	n/a	14320

*analyte peak was not able to quantified.

Table C-3. Extraction Concentrations Prior to Blank Correction and Surrogate Recovery

	Uncorrected Extraction Concentrations (µg/L)*							Surrogate recovery	
Sample ID	Fluorene	Phen-anthrene	Anthra-cene	Fluor-anthene	Pyrene	Benzo(a) anthracene	Chrysene	d-10 recovery (%)	d-12 recovery (%)
Ak 1260.2	n/a**	503.5	76.1	138.9	40.1	n/a	n/a	101	0
Rich 109.1	n/a	317.9	7.9	88.6	81.8	16.4	87.8	96	62
Dal 13.1a	n/a	270.3	9.4	56.7	32.1	5.4	10.4	75	224
Dal 13.1b	n/a	349.6	7.4	46.4	53.8	8.4	21.4	106	217
Sew 8.2a	51.0	318.7	20.2	n/a	115.3	13.2	79.5	88	70
Sew 8.2b	106.9	588.8	40.2	151.9	175.6	23.8	123.0	111	91
Dal 160.2a	99.9	346.7	16.0	51.9	42.4	5.6	238.8	69	57
Dal 160.2b	116.8	238.1	6.8	47.9	88.7	18.5	258.7	78	49
Nov18 blank	80.8	145.9	6.8	29.6	80.8	9.4	14.6	103	94
Ak 1301.1a	184.5	772.0	28.3	112.1	120.3	12.9	78.7	69	59
Ak 1301.1b	54.4	138.6	4.3	32.8	31.5	4.2	52.2	96	77
Ak 1301.2a	62.1	243.1	18.8	62.2	85.0	20.1	72.8	112	77
Ak 1301.2b	114.8	368.5	8.9	69.3	55.1	5.0	35.8	85	71
Nov19 blank	47.1	202.2	10.8	37.9	37.6	2.0	10.6	95	92
Dal 160.1a	134.3	289.8	9.6	70.4	57.9	n/a	430.4	88	79
Dal 160.1b	138.1	268.2	10.9	65.9	93.2	6.1	315.7	97	67
Rich 207.2a	106.4	329.3	13.0	108.5	136.3	9.7	64.1	123	84
Rich 207.2b	58.7	194.9	5.0	49.2	42.2	1.7	8.1	98	109
Ell 9.1a	72.6	215.2	5.8	55.6	51.3	3.9	30.5	118	109
Ell 9.1b	69.0	231.4	4.3	48.7	54.6	6.4	38.4	80	68
Rich 109.2	41.7	345.9	21.5	62.4	44.7	6.2	31.5	72	60
Dal 13.2a	38.0	148.3	2.6	40.2	27.2	2.2	25.0	105	92
Dal 13.2b	62.8	208.9	10.5	12.2	39.8	3.0	28.5	79	69
Rich 339.2a	66.8	337.8	4.6	204.0	150.3	18.4	88.4	86	66

Table C-3. (con't)

	Uncorrected Extraction Concentrations (µg/L)*							Surrogate recovery	
Sample ID	Fluorene	Phen-anthrene	Anthra-cene	Fluor-anthene	Pyrene	Benzo(a) anthracene	Chrysene	d-10 recovery (%)	d-12 recovery (%)
Rich 339.2b	68.2	358.3	12.1	182.7	121.4	20.3	82.3	85	63
Sew 75.2a	47.7	168.5	8.5	60.3	41.7	6.7	38.5	108	96
Rich 309.1sp	477.3	688.3	419.3	471.2	473.4	408.1	524.5	94	84
Rich 309.1	65.0	232.5	7.4	48.4	64.9	14.1	55.7	84	71
Sew 75.2b	50.7	264.0	20.6	53.9	54.0	6.6	46.4	136	108
Rich 31.1a	44.8	153.9	7.0	34.1	29.1	4.3	23.9	83	67
Rich 31.1b	50.7	136.7	6.8	31.1	26.0	3.1	17.9	91	86
Dec12 blank	39.0	149.1	6.4	23.4	20.9	n/a	6.5	75	77
Dec10 blank	36.2	130.5	5.4	21.3	17.3	4.5	6.1	78	81
Jan29 blank	42.1	97.6	3.9	17.5	42.6	n/a	7.1	107	114
Rich 339.1b	142.6	730.6	10.1	361.0	228.8	45.0	360.5	88	85
Rich 31.2a	66.5	224.5	5.4	45.4	69.1	5.1	24.6	92	68
Rich 31.2b	69.1	170.7	9.3	41.9	65.9	19.9	6.0	92	87
Feb3 blank	40.2	165.6	9.1	27.8	26.5	n/a	6.3	84	85
Ell 47.1	n/a	229.5	2.2	36.5	30.4	6.7	155.0	80	62
Ell 47.1sp	n/a	570.7	238.1	278.0	256.1	228.2	377.9	76	61
Ak 1418.1	78.4	312.2	8.4	59.6	47.6	2.9	21.7	74	62
Ak 1418.1sp	298.8	515.9	250.3	291.8	287.7	239.7	232.1	87	79
Park 67.1	66.2	275.5	14.8	96.3	84.4	6.5	46.4	98	98
Park 147.1a	101.7	294.2	9.7	60.0	86.6	18.3	35.4	97	81
Park 147.1b	81.4	236.0	13.3	53.4	43.2	3.8	23.8	84	75
Park 67.2	59.1	230.0	11.6	77.8	72.1	6.0	54.0	94	75
Ell 9.2a	61.4	223.7	11.4	71.7	56.9	4.9	31.9	87	80
Ell 9.2b	65.6	216.9	11.1	62.3	60.4	3.4	32.0	74	67
Feb11 blank	52.9	176.3	6.5	30.4	29.0	2.3	9.1	80	80
Feb12 blank	36.6	141.6	4.5	23.6	28.2	n/a	7.3	87	89
Park 147.2a	75.3	170.2	2.8	33.6	48.9	5.2	84.8	87	71
Park 147.2b	58.6	163.1	3.6	35.7	36.6	4.3	68.1	72	62
Ak 1418.2	90.9	193.2	4.6	45.5	42.2	3.6	112.8	88	77
Ak 1418.2sp	339.9	392.2	249.8	269.1	270.8	231.4	307.4	36	35
Dal 193.1	34.6	173.8	7.8	34.6	27.4	2.7	45.4	110	99
Feb13 blank	32.6	68.1	1.5	13.7	43.5	4.2	5.4	105	117
Dal 193.1sp	246.0	387.3	236.3	256.3	268.5	249.3	279.8	86	70
Ak 1260.1	51.6	331.2	26.4	69.6	106.3	n/a	n/a	79	0
Dal 193.2	28.5	105.2	3.0	22.7	18.9	2.4	9.1	82	69
Dal 193.2sp	255.9	384.3	243.9	239.9	254.4	269.4	274.5	85	64
Rich 339.1a	6.6	884.8	16.3	422.7	318.3	40.7	480.7	97	71
Feb18 blank	119.2	313.3	4.6	64.1	54.7	n/a	9.1	15	15
Dal 87.1	79.0	235.0	3.9	40.3	80.0	1.2	16.3	85	72
Rich 16.1	56.5	108.1	n/a	29.3	54.7	n/a	23.4	175	211

Table C-3. (con't)

	Uncorrected Extraction Concentrations (µg/L)*							Surrogate recovery	
Sample ID	Fluorene	Phen-anthrene	Anthra-cene	Fluor-arthene	Pyrene	Benzo(a) anthracene	Chrysene	d-10 recovery (%)	d-12 recovery (%)
Rich 16.1sp	140.0	391.3	250.1	268.0	256.1	220.9	257.5	98	113
Sew 42.2	52.4	198.1	7.1	44.1	31.8	4.0	38.1	102	87
Sew 42.2sp	276.3	388.1	244.8	273.5	242.4	255.5	269.4	72	59
Feb20 blank	26.2	91.8	2.9	16.6	18.5	n/a	4.5	106	112
Rich 277.1	76.1	200.2	2.8	56.5	104.8	4.4	27.8	92	81
Sew 42.1	68.3	204.6	10.1	48.1	37.2	5.4	57.4	88	79
Sew 42.1sp	258.1	396.4	242.7	254.8	246.7	239.5	254.5	75	64
Rich 309.2	54.5	217.5	2.5	60.3	106.6	9.7	59.6	99	86
Rich 309.2sp	264.5	441.0	252.3	315.2	307.4	257.8	286.5	74	59
Rich 16.2	99.2	225.9	6.0	36.1	27.2	3.2	84.3	104	108
Rich 16.2sp	319.8	381.7	249.9	285.0	263.5	280.5	292.6	69	51
Feb26 blank	12.3	34.6	0.9	7.7	6.6	n/a	2.2	74	73
Rich 277.2	67.4	100.8	2.8	41.9	39.9	4.3	147.6	84	89
Park 230.2	50.7	161.6	n/a	40.5	39.7	4.2	35.0	63	56
Park 230.2sp	288.0	380.3	241.7	306.7	291.3	287.8	235.8	76	56
Park 214.1	22.8	114.1	0.6	7.2	32.9	3.5	28.2	75	59
Park 214.2	43.8	164.5	3.8	39.0	34.7	3.0	52.9	58	53
Ak 1384.1	26.9	106.8	3.2	22.1	21.1	3.5	51.0	78	73
Ak 1384.2	39.7	130.0	n/a	25.8	28.5	4.4	41.7	90	84
Park 270.1a	3.5	353.8	6.5	54.9	44.4	n/a	225.9	54	44
Park 270.1b	3.6	342.9	3.5	60.7	45.5	5.2	187.1	102	59
Mar3 blank	25.1	56.6	1.5	10.0	14.9	0.7	2.2	65	65
Ak 1230.2	27.6	133.6	1.9	22.0	22.3	2.8	35.9	58	46
Ak 1230.2sp	237.4	376.4	241.7	240.7	250.3	246.6	288.9	60	53
Ak 1230.1	17.2	114.2	n/a	11.2	21.6	2.3	103.5	72	65
Ak 1230.1sp	281.3	388.7	256.0	274.4	253.5	254.8	330.5	94	91
Dal 87.2	31.9	100.6	n/a	16.4	24.0	n/a	58.6	52	43
Rich 72.2	27.2	111.8	1.7	15.2	16.6	1.3	95.1	61	54
Dal 122.2	5.2	74.0	3.2	13.4	10.9	n/a	196.0	44	38
Dal 50.1	n/a	123.3	5.8	9.5	12.7	n/a	587.4	76	0
Dal 50.1sp	n/a	380.6	222.8	209.2	232.3	238.2	775.5	59	33
Sew 8.1a	84.7	149.8	3.0	56.9	42.3	5.8	46.5	60	66
Sew 8.1b	61.5	139.2	2.7	54.3	32.4	6.7	46.0	72	64
Mar10 blank	40.5	137.6	2.3	24.9	18.1	1.8	3.2	82	81
Rich 72.1	46.7	222.9	1.0	31.9	25.3	n/a	17.8	71	52
Dal 50.2	76.0	253.7	0.6	44.9	27.4	n/a	75.4	70	55
Dal 50.2sp	270.2	509.8	257.5	236.9	255.5	254.3	280.3	40	24
Park 107.2	55.3	265.6	1.0	47.4	30.8	2.3	196.9	63	41
Park 107.2sp	335.2	644.9	254.1	333.1	281.4	307.9	455.9	52	32
Rich 4.1	n/a	163.5	2.8	71.2	84.7	n/a	n/a	59	0

Table C-3.(con't)

	Uncorrected Extraction Concentrations (μg/L)*							Surrogate recovery	
Sample ID	Fluorene	Phen-anthrene	Anthra-cene	Fluor-arthene	Pyrene	Benzo(a) anthracene	Chrysene	d-10 recovery (%)	d-12 recovery (%)
Rich 4.2	19.8	253.7	0.6	44.9	27.4	n/a	75.4	80	0
Dal 122.1	44.7	159.0	2.5	7.8	42.2	n/a	269.8	77	54
Rich 166.2	45.3	154.2	n/a	5.3	18.4	n/a	155.5	80	58
Rich 166.2	250.2	442.8	256.7	240.8	286.0	198.2	348.2	54	31
Park 187.1	31.5	164.5	n/a	26.1	18.2	n/a	56.1	76	50
Mar11 blank	39.4	134.9	1.3	23.4	16.4	1.0	2.8	77	72
Park 187.2	59.7	173.8	2.3	49.6	67.7	2.5	34.7	70	60
Ak 1345.1	62.4	185.0	2.6	45.3	62.8	n/a	49.9	73	43
Ak 1345.2	12.8	130.6	1.8	34.1	62.1	1.9	28.6	79	54
Park 107.1	25.0	111.5	2.1	28.7	24.4	2.9	31.6	93	60
Park 107.1sp	283.6	433.1	247.1	260.4	315.3	279.6	264.3	88	66
Rich 166.1	44.2	173.2	n/a	30.0	55.4	n/a	11.3	67	44
Rich 166.1sp	292.7	389.9	250.6	243.6	276.6	308.6	271.6	72	49
Park 230.1	59.2	213.1	2.6	54.7	41.7	1.7	20.4	77	55
Park 230.1sp	303.8	494.2	248.3	278.4	306.9	351.4	281.3	68	47
Park270.2a	n/a	267.1	6.2	49.8	89.7	n/a	346.0	75	48
Park 270.2b	n/a	355.5	7.1	58.2	82.7	n/a	395.8	51	32
Ell 47.2	144.2	435.1	5.2	56.9	87.0	8.6	391.66	34	22
Ell 47.2sp	295.3	427.5	261.6	236.2	264.1	217.7	450.37	49	38
Gold 1	95.8	558.4	7.1	249.2	171.8	11.5	120.8	44	36
Gold 2	66.3	344.4	3.8	222.5	189.7	11.0	114.4	81	56
Apr7 blank	25.9	86.5	1.0	13.3	10.1	n/a	2.5	84	90
Nov26 blank	40.0	166.4	4.9	28.8	25.3	n/a	n/a	69	71

*Uncorrected refers to extraction concentrations without blank correction.

**no data obtained.

Table C-4. Benzo(a)anthracene and Chrysene Average Site Concentrations

Sample ID	Benzo(a)-anthracene	Chrysene	Sample ID	Benzo(a)-anthracene	Chrysene
	(ng /g dry needle)			(ng /g dry needle)	
Ak 1230	1.2	20.2	Park 187	1.2	5.9
Ak 1260	n/a*	n/a	Park 214	n/a	n/a
Ak 1301	2.0	9.2	Park 230	1.5	5.2
Ak 1345	n/a	n/a	Park 270	n/a	n/a
Ak 1384	1.5	8.6	Rich 4	n/a	n/a
Ak 1418	1.2	12.7	Rich 16	1.4	16.5
Dal 13	0.8	2.7	Rich 31	2.1	1.9
Dal 50	n/a	n/a	Rich 72	n/a	n/a
Dal 87	1.0	2.7	Rich 109	1.3	4.7
Dal 193	1.2	4.8	Rich 166	n/a	n/a
Dal 160	0.6	74.0	Rich 207	1.3	6.3
Dal 122	n/a	n/a	Rich 277	1.7	17.5
Ell 9	1.3	5.5	Rich 309	2.9	10.5
Ell 47	1.8	28.6	Rich 339	6.2	46.5
Goldstream	n/a	n/a	Sew 8	3.0	14.5
Park 67	2.0	10.0	Sew 42	1.7	9.2
Park 107	1.4	5.6	Sew 75	1.6	7.5
Park 147	2.4	10.2			

*Data either unobtainable from chromatograms or d12-surrogate recovery outside of range, 60-140%.

Table C-5. Peak Areas for GC/MS Standards.

Sequence date	Std ID	Fluorene	Phen-anthrene	Anthra-cene	Phen-anthrene d-10	Fluor-anthene	Pyrene	Benzo(a)-anthracene	Chrysene	Chrysene d-12
11-Feb	a1	47788	59919	57514	37822	58114	59098	40892	35368	22797
11-Feb	a2	35007	43728	44326	27959	40434	39224	19693	19195	12010
11-Feb	b1	15248	20090	20344	13830	19962	19158	12043	10847	7694
23-Feb	a1	41822	48696	48366	32540	49227	50900	33516	32556	21074
23-Feb	a2	37474	44221	44762	29913	43081	43391	29024	26953	17566
23-Feb	b1	37380	44132	43981	29865	43552	44762	28978	27786	18431
23-Feb	b2	34095	41124	41784	27705	39432	39930	25683	23665	15917
25-Feb	a1	59133	68077	67337	44175	70037	71633	34112	32782	28104
25-Feb	a2	49563	44221	44762	29913	43081	43391	28778	26188	17566
25-Feb	b1	41551	47432	46302	30828	47958	49395	29449	27882	18130
25-Feb	b2	47585	54586	55426	35777	53842	52312	30348	27643	18186
27-Feb	a1	55604	70190	70039	42807	68561	71706	50254	48128	29587
27-Feb	a2	46232	58372	59618	36702	55912	53097	32784	30271	20590
27-Feb	b1	52606	65951	66028	40754	63497	65088	42525	39584	25126

Table C-5. (con't)

Sequence date	Std ID	Fluorene	Phen-anthrene	Anthra-cene	Phen-anthrene d-10	Fluor-anthene	Pyrene	Benzo(a)-anthracene	Chrysene	Chrysene d-12
27-Feb	b2	50893	62910	64159	38585	56836	56555	31867	28186	17662
2-Mar	a1	50314	61874	61592	41319	59551	59446	36479	34491	24494
2-Mar	a2	42896	53631	53907	36552	49687	49567	30801	28738	19690
2-Mar	b1	46206	56412	56559	38556	52923	54685	32843	30828	21473
2-Mar	b2	42101	53022	53581	35840	49346	49866	29154	26858	18052
4-Mar	a1	39513	49071	49192	30162	46523	48466	30585	28932	18982
4-Mar	a2	34435	42852	43634	27181	40723	41997	26832	24789	16113
4-Mar	b1	36793	43715	43242	28283	43113	44634	26187	24822	17632
4-Mar	b2	36009	45853	46419	27933	43349	44892	27055	25806	16032
8-Mar	a1	79903	98344	98396	67364	106031	106444	74399	68897	40936
8-Mar	a2	66361	78865	79734	54054	80386	80705	50952	46735	28567
8-Mar	b1	65675	77718	77152	54577	81824	82071	51104	47584	29619
8-Mar	b2	59787	71263	71572	49185	71863	72176	42708	39115	23927
11-Mar	a1	66904	109011	108766	69704	107027	107648	76236	70931	42850
11-Mar	a2	66904	85648	86299	54386	78653	79253	52625	48700	30064
11-Mar	b1	74397	94199	93797	61675	97047	100246	64624	60961	39045
11-Mar	b2	68816	87312	88560	55508	78321	78213	48968	45304	27651
1-Apr	a1	81656	98603	93283	71793	114863	117111	64338	62173	36091
1-Apr	a2	69940	88848	88549	60480	86613	86035	48464	44712	25589
1-Apr	b1	80564	98832	98017	71095	110300	114479	64271	59479	34705
1-Apr	b2	59290	74994	74895	52216	69953	67196	35714	32478	18841
3-Apr	a1	101876	123614	122086	83897	131113	135200	78568	74620	48441
3-Apr	a2	100337	125781	125160	83761	120583	120199	64041	60799	39016
3-Apr	b1	115717	135783	133178	93067	148373	151533	82920	83328	54133
3-Apr	b2	118615	145150	143965	96956	139028	139343	81548	82173	40783
8-Apr	a1	87249	106312	104640	80102	116819	119778	72530	70591	44634
8-Apr	a2	89955	111876	112707	80082	107377	105916	60546	56078	33337
8-Apr	b1	99690	114895	113210	88826	122604	129931	75213	73110	44559
8-Apr	b2	96508	122519	123548	87537	112342	110010	59074	53874	31775
9-Apr	a1	75360	86882	85685	66907	95244	96784	49571	45337	32447
9-Apr	a2	75573	90796	91084	67021	88862	85097	40315	37720	25668
9-Apr	b1	80804	92634	92102	72906	99829	99004	47895	45482	30771
9-Apr	b2	72731	87215	87887	65082	80491	78301	34898	32222	22861
13-Apr	a1	88105	94036	90465	72747	114822	117784	50967	55864	41413
13-Apr	a2	87361	99586	99417	71624	107469	106747	49946	45138	32262
13-Apr	b1	95963	102134	99206	81108	122984	126942	57737	53594	42804
13-Apr	b2	94188	105925	104919	76842	113276	113053	55885	50152	34458

Appendix D. Sample Concentration Calculation.

Step 1. Calculate average standard signal.

Standard a1 signal = std peak area / internal std peak area

Repeat for a2 and average both signals

Step 2. Calculate Calibration Factor (slope from one-point calibration curve)

Cal. Factor = avg std signal / conc of std (250 μg/L)

Use this Cal Factor for samples that were analyzed between stds a1 & a2 in that sequence.

Step 3. Calculate Sample Signal.

Sample signal = sample peak area / internal std peak area.

Step 4. Calculate Raw Extract Concentration

Raw ext. conc. (μg/L) = sample signal / cal factor

Step 5. Blank Correct Raw Extraction Concentration.

Blank correction factor (μg/L) = method blank slope (μg/L/day #) * extraction day #(day #) + method blank y-intercept (μg/L)

Blk Corr. Ext. Conc. (μg/L) = Raw ext. conc. (μg/L) – blk corr. Factor(μg/L)

Note: blank correcting the data resulted in a few negative concentrations. Concentration were adjusted to be greater than zero by adding the absolute value of the minimum concentration of analyte to all concentrations for that analyte.

Step 6. Calculate Needle Concentration.

Needle conc. (ng/g dry needle) = blk corr ext. conc (μg/L) * volume of extract (L) / dry mass of needles (g)

Volume of extract = 250 μL or 2.5×10^{-4}L

Dry mass of needles (g) = mass of needles (g) * (100- % water)/100

Appendix E. Parameters Used for PLS Models.

Parameters for PLS-1 models for phenanthrene, anthracene, fluoranthene and pyrene

Software: The Unscrambler 6.11a by CAMO ASA

Calibration Method: PLS1

Validation Method: leverage correction

Number of Calibration Samples: 35

Number of Validation Samples: 0

Data is centered

Number of PCs Calculated: 4

Sample Set: All Samples [35]

Excluded Samples: See Table 4.7

Sample Weights: All 1.0

X-Variable Set: All Variables [18]: latitude, longitude, elevation, radial distance from urban site, 3 species, 7 ecosystem, temperature, precipitation, non-volatile extractable content and potential forest fire impact.

X-Variable Weights: All 1/SDev

Y-Variable Set: Single analyte [4]: phenanthrene, anthracene, fluoranthene, or pyrene.

Y-Variable Weights: All 1/Sdev

Parameters for PLS-2 models for 3-ring and 4-ring PAHs.

Software: The Unscrambler 6.11a by CAMO ASA

Calibration Method: PLS2

Validation Method: leverage correction

Number of Calibration Samples: 20

Number of Validation Samples: 0

Data is centered

Number of PCs Calculated: 4

Sample Set: See Table 4.10.

Excluded Samples:

AK 1260, Rich 339, Goldstream.

Sample Weights: All 1.0

X-Variable Set: All Variables [18]: latitude, longitude, elevation, radial distance from Fairbanks, 2 species, 5 ecosystem, temperature, precipitation, non-volatile extractable content, potential forest fire impact, direction from Fairbanks (3).

X-Variable Weights: All 1/SDev

Y-Variable Set: 3-ring [2]: phenanthrene and anthracene

4-ring [2]: fluoranthene and pyrene.

Y-Variable Weights: All 1/Sdev

Excluded Y data: phenanthrene: AK 1301, AK 1418, Parks 270

anthracene: AK 1301, AK 1418, Elliot 47

fluoranthene: Dalton 122, Dalton 160

pyrene: Dalton 87, Dalton 122